REPORT OF THE WORK DONE

INTRODUCTION

The Myristicaceae is a family of arborescent flowering plants that have significant ecological and ethno-botanical importance worldwide in wet lowland forests. The Western Ghats inhabits five species of Myristicaceae belonging to three genera viz. *Myristica, Knema* and *Gymnacranthrea*; all of them are trees associated with evergreen to semi evergreen forests. Among five species of Myristicaceae, *Myristica fatua var. magnifica* and *Gymnacranthera canarica* are exclusive to the swamps. A myristica swamp was reported for the first time as a special type of forest formation by Krishnamoorthy (1960) from Travancore in Southern Kerala.

Myristica fatua var. magnifica, known as Daasapathre (Kananda) is a large tree, grows up to 30 m height and often buttressed and furnished with numerous aerial roots. The fruit is oblong and densly tomentose with orange red aril (Gamble 1967). In Karnataka, it is reported from Gerusoppa, Kathalekan forest of Uttara Kannada district (Chandran 1990) and Hulikal Ghat of Shimoga district (Dasappa & Ram 1999). The red arils from seeds are used for dyeing purpose. The seeds yield oil that is used for burning and making candles (Anonymous 1962; Gamble 1967; Hooker 1973). *Gymnacranthera canarica* is a large evergreen tree, with numerous stilt roots. The fruit is globose with thick fleshy pericarp (Gamble 1967). It is mostly distributed in and around the unique fresh water marshy ecosystems called 'Myristica swamps'. Seed fat is used for the preparation of candles (Mabberley 1990). The fat extracted from seed and aril is used in soap making (Anonymous 1962).

M. malabarica, known as Kanaje (Kannada), Bombay mace (English), Ponnam panu (Malayalam) and Patthiri (Tamil) is a tree reaching up to a height of 15 m. The ripe nutmeg and mace of *M. malabarica* are used as adulterants of *M. fragrans*. Gokhale et al. (2004) reported that mace is used as cooling agent, febrifuge and expectorant while seed fat is good for myalgia, sprains and sores. *M. dactyloides,* commonly known as Kaadu ramapthri (kannada), Kathu jayikayi (Tamil) is also frequently found in the evergreen forests. It produces globose fruits with orange red aril (Gamble 1967). Seed paste prepared with water is administered orally to cure

dysentery. *Knema attenuata* also known as Raktha mara (Kannada), Chora patthiri (Tamil), Chora panu (Malayalam) is a moderate sized tree endemic to Western Ghats. The fruit is ovoid with brilliant crimson red coloured aril. The plant is known for its medicinal importance and also serves as one of the ingredients of 'Ashwagandadhi nei' (medicated ghee) used for the treatment of multiple ailments in Ayuveda (Ravi kumar & Ved 2000).

Phytochemical studies

Preliminary phytochemical screening

Subha et al. (2013) reported the presence of alkaloids, tannins, saponin, flavonoids, amino acid, flavonoids, steroids, glycosides and carbohydrates in the leaf extracts of *M. dactyloides* during the preliminary investigations. Presence of alkaloids, glycosides, steroids was confirmed by thin layer chromatography.

Proximate Composition

Anonymous (1962) reported 6.9% moisture, 40.76% fat and resin, 6.5% protein, 42.18% carbohydrate, 2.33% fibre and 1.33% ash content in the Kernel and 4.07% moisture, 63.26% fat and resins, 7.31% protein, 20.80% carbohydrate, 3.06% fibre and 1.50% of ash content in the mace of *M. malabarica*. Rama Bhat & Kaveriappa (1998) reported the proximate composition in the mace and kernel of *M. fatua* with a higher protein content (14.1, 11.6%), starch (35, 59%), ash (6.3, 5.5%) and tannin content (8.9, 8.5%) compared to the mace and kernel of *M. fragrans*.

Amino acid composition

Maya et al. (2006) studied the amino acid composition of leaves of Myristica wild species using HPLC technique. According to them *M. magnifica* leaf had lowest (127.65µg/100mg) and *M. prainni* (215µg/100mg) highest leaf amino acid content. Phenyl alanine (119.6 µg/100mg), alanine (60.7µg/100mg), valine (34.8 µg/100mg), tyrosine (19.9µg/100mg), threonine (18.2µg/100mg) were the major amino acids in the leaves of *M. malabarica* while *M. fatua* leaf contained alanine, tyrosine, valine and threonine with 49.1, 33.6, 26.6 and 11.3µg/100mg respectively.

Fatty acids

Maya et al. (2006) studied the fatty acid contents of wild taxa of *Myristica fragrans* and reported a good amount of fat content (29.54%) from mace of *M. malabarica*. Stearic acid was the dominant fatty acid in kernel (57.64%) and mace (59.39%) of *M. malabarica.* Myristic acid (19.06%), palmitic acid (1.34%), lauric acids (0.49%) were the major fatty acids of kernel while myristic acid (3.22%) and palmitic acid (2.23%) were found in mace of *M. malabarica*.

Sreenivasan (1968) studied nineteen different samples of oils and fats and reported the presence of more unsaturated fatty acids with myristic, palmitic and oleic acid as their major components in *K. attenuata, M. magnifica*. *M. canarica* showed saturated fatty acids (80%) having more than 90% of myristic acid and lauric acid. *M. canarica, M. attenuata* and M. *fatua var. magnifica* fat contained 20%, 40%, and 80% of resinous and phenolic matter respectively.

Leaf essential oil

Sabulal et al. (2007) reported 76 constituents with β-caryophyllene(27.3%),α-humulene(13.8%), α- copaene (11.5%) as major components from the leaf of *M. malabarica*. Also, 76 constituents (98.1%) with β-caryophyllene (23.4%), linalool (13.4%) and α-humulene (11.3%) as major constituents of leaf essential oil of *Gymnacarnthera canarica*. According to Zachariah et al, (2008) *M. malabarica* leaf essential oil is dominated with sesqiterpenes (73%), β-caryophyllene (20.15%), α-humulene (10.17%), nerolidol(9.25%) and δ-cadinene(6.72%).

Diarylnonanoids

Four diarylnonanoids and malabaricones A-D were isolated from the fruit rind of *Myristica malabarica* Lam. by Purushothaman et al. (1977). Patro et al. (2005) isolated a new 2-acylresorcinol and four known diarylnonanoids (Malabaricone A-D) from *M. malabarica*.

Flavonoids

Photochemical investigations of the heartwood of the *Myristica malabarica* lead to the isolation of the new 7,4-dimethoxy -5-hydroxyisoflavone along with the

two other isoflavones, Biochanin A and prunetin and a rare α-hydroxydihydrochalcone as reported by Talukdar et al. (2000).

Phenolic compounds

Three antifungal resorcinols (Malabaricones –A, B, C) were isolated from the methanol extract of *M. malabarica* fruit rinds (Choi 2008). Vinayachnadra and Chandrashekar studied the phenolic contents in ethanolic extracts of seed and aril of *Knema attenuata* fruits (2014) and noticed the presence of phenolic compounds like quercetin (0.51 & 0.22mg/100mg), gallic acid (0.0016 & 0.0010 mg/100mg), ferrulic acid (0.0010 & 0.0004mg/100mg) and catechin (0.0057 & 0.0006 mg/100mg) in ethanolic extracts of seed and aril. Cooray et al. 1987 isolated and reported six acylresorcinols namely 1-(2, 6-dihydroxyphenyl)-9-(4-hydroxy-3-methoxyphenyl) nonan-1-one, 1-(2, 6-dihydroxyphenyl) tetradecan-1-one and malabaricones A-D from seeds of *M. dactyloides*.

Chemical investigation of the hot hexane extract of the stem bark of *Myristica dactyloides* has resulted in the isolation of two new lignans, rel-(8*S*,8′*R*)-dimethyl-(7*S*,7′*R*)-bis(3,4-methylenedioxyphenyl)tetrahydrofuran and rel-(8*R*,8′*R*)-dimethyl-(7*S*,7′*R*)-bis(3,4-methylenedioxyphenyl)tetrahydrofuran, a new diaryl alkanone, 1-(2,6-dihydroxyphenyl)-9-(4-hydroxy-3-methoxyphenyl)nonan-1-one, sitosterol and six other previously reported aryl alkanones (Herath & Priyadarshani, 1996). Herath and Priyadarshini (1997) reported a new lignan, rel.(8S,8'S)- bis(3,4-methylenedioxy)-8,8'-neolignan from the hexane extract of stem bark of *Myristica dactyloides*. Also, chloroform extract of stem bark resulted two more lignans, rel.(8S,8'R)dimethyl-(7S,7'R)-bis(4-hydroxy-3-methoxyphenyl) tetrahydrofuran and rel. (8S,8'S)dimethyl-(7S,7'S)-bis(4-hydroxy- 3-methoxyphenyl) tetrahydrofuran.

A new lignin, attenuol (58%) was isolated from the hexane extract of bark of *Knema attenuata* (Joshi et al. 1977). Kumar et al. (1988) reported four new aryl alkanones from the stem bark of *M. dactyloides* namely 1-(2-methoxy-6-hydroxyphenyl)tetradecan-1-one , 1-(2-methoxy-6-hydroxyphenyl)- 9-(3´,4´-methylenedoxyphenyl)-nonan-1-one, 1-(2,6-dihydroxyphenyl) tetradecan-1-one and 1-(2-methoxy-6-hydroxyphenyl)-9-(4´-hydroxyphenyl)-nonan-1-one.

Other compounds

Gokhale et al. (2004) determined the chemical constituents of false nutmeg (*Myristica malabarica*) and reported the presence of myristic, palmitic and oleic acid. Tillekeratne et al. (1981) reported Myoinositol, a hexitol from the methanolic bark extract of *M. dactyloides.*

Pharmacological studies

Antioxidant activity

The phenolic compounds present in the seed resin of *M. malabarica* were found to be good antioxidants in various edible oils and fats more efficiently than butylated hydroxytoluene as reported by Duggal & Kartha (1956). Patro et al. (2005) isolated a new 2-acylresorcinol and four known diarylnonanoids (Malabaricone A-D) from *M. malabarica*, of which diaryllnonanoid, malabaricone C showed the maximum DPPH scavenging activity. Also, they found that Malabaricone C could prevent both Fe (II) - and 2, 2' azobis (2-amidinopropane) dihydrochloride induced lipid peroxidation of rat liver mitochondria more efficiently compared to curcumin. Ajish et al. (2015) reported DPPH radical scavenging activity of petroleum ether extracts at 10µg/ml in *M. dactyloides* bark.

Viveka & Chandrashekar (2016) studied the antioxidant property of *M. fatua* and reported the significantly good antioxidant activities of different solvent extracts of *M. fatua* bark, kernel, aril and fruit rind. Ethyl acetate extract of testa exhibited a good response to the DPPH activity by showing higher IC_{50} at 0.0315 mg. The benzene fraction of seed aril of *M. malabarica* showed significant DPPH scavenging activity and that was comparable with standard ascorbic acid as reported by Manjunatha et al. (2011). In *Knema attenuata*, chloroform extract of aril showed higher activity in DPPH radical scavenging, reducing power assay and hydrogen peroxide scavenging as reported by Vinayachandra & Chandrashekar (2014).

Methanolic extract of stem bark of *M. malabarica* showed a significant dose dependent antioxidant activity with IC_{50} values at 0.02mg/ml in DPPH assay, 0.107mg/ml in scavenging of hydrogen peroxide, 1.6µg/ml in ABTS radical cation decolourisation assay and 0.5mg/ml in Nitric oxide scavenging assay as reported by Manjunatha et al, (2012). Petroleum ether extract of *M. dactyloides* bark showed a

potent DPPH scavenging activity at the concentration of 10µg/ml as reported by Vagdevi et al. (2015). Methanolic extracts of *Myristica malabarica* showed high superoxide scavenging activity (Khanom 2000).

Gastric ulcer

Chattopadyaya et al, (2007) studied the healing property of methaolic extract of *M. malabarica* against indomethacin induced stomach ulceration and reported that upon treatment with 40mg/kg/day of the extract for 3 days showed 72% healing effect. Malabaricone B and Malabaricone C are the major antioxidant constituents of *M. malabarica* which showed significantly good healing property (60.3%& 88.4%) against indomethacin induced gastric ulceration in mice (Chattopadyaya et al, 2008).

Larvicidal activity

Vinayachandra et al, (2011) reported the larvicidal activity of *Knema attenuata* kernel and aril extracts against *Aedes albopictus* Skuse and *Anopheles stephensi* Liston. Chloroform extracts of aril showed 100% mortality against both larval forms of *A. albopictus* and *A. stephensi* at the concentration of 500 ppm.

Hepatoprotective activity

Majunatha et al, (2011) reported the hepatoprotective activity of *Myristica malabarica* aril extracts. The benzene fraction showed a significant decreae in total bilirubin, alanine transaminase, aspartate transaminase, alkanine phosphatase and an increase in total protein content indicating the potency of benzene fraction against carbon tetrachloride induced hepatic damage. Manjunatha et al, (2012) also reported the hepatoprotective activity of methanolic extract of stem bark of *M. malabarica* and proposed that hepatoprotection against carbon tetrachloride induced hepatic damage might be due to its active constituents like Biochanin A, Malabaricone C, Malabaricone A, Malabaricone B and Malabaricone D through in silco study.

Antiparasitic activity

Julie et al, (2007) studied the antiparasitic activity of some New Calodonian medicinal plants and reported that aril extracts from *M. fatua* exhibited an IC_{50} value of 0.8µg/ml against *Trypanosoma brucei* while kernel extract presented significantly

good antiparasitic activity against *Trypanosoma brucei* (IC_{50} value at 5µg/ml) *and Caenorhabditis elegans* (IC_{50} value at 6.6µg/ml).

Antimicrobial activity

Viveka & Chandrashekar (2016) reported a good antibacterial activity by the chloroform extract of *M. fatua var. magnifica* aril against *Staphylococcus aureus* with the minimum bactericidal concentration of 5mg/ml. Vagdevi et al, (2015) studied antimicrobial activities of *M. dactyloides* bark and reported the susceptibility of *Trichoderma viridae* with an inhibition zone of 25.90mm in the petroleum ether extract. Koperuncholan et al. (2011) reported antibacterial and antifungal activities in petroleum ether, chloroform, and ethanol extracts of root bark, stem bark and leaves of *M.dactyloides.* Vinayachandra Chandrashekar (2014) reported highest antibacterial activity of chloroform extract of aril and hexane extract of *Knema attenuata* seed against *Staphylococus aureus* with MIC of 12563µg/ml and 12541µg/ml respectively.

In *in-vivo* antifungal assay, all the three resorcinols (Malabaricones- A, B and C) isolated from *M. malabarica* fruit rind effectively suppressed the development of rice blast, wheat leaf rust and red pepper anthracnose as reported by Choi et al. (2008). Also, they reported the inhibitory potential against mycelial growth of eight plant pathogenic fungi including *Alternaria alternata* (IC_{50}@22µg/ml), *Colletotrichum gloeosporioides* (IC_{50}@17µg/ ml), *Rhizoctonia solanii*(IC_{50} @36µg/ ml) towards Malabaricone B. This was the first report of antifungal property of malabaricones against filamentous fungi.

Anthelmintic activity

Bark petroleum ether extract of *M. dactyloides* exhibited dose dependent anthelmintic activity as compared to standard drug Albendazole (Vagdevi et al. 2015). The mean paralyzing time of *Pheretima Posthuma* with the dose of 50 and 100 mg/ml for petroleum ether extract were found to be 22.83±0.09 and 10.97±0.15 minutes respectively.

Other pharmacological studies

Choi et al. (2008) reported 100% mortality of nematode, *Bursaphelenchus xylophilus* by methanolic extract of *M. malabarica* fruit rind at the concentration of 1000µg/ml. Malabaricones isolated from *M. malabarica* showed antipromastigote property (Sen et al. 2007) .

MATERIALS AND METHODS

Study area and collection of samples

Mature, split and aril exposed fruit, leaf and bark of *Myristica dactyloides* Gaertn. was collected directly from Dakshina Parse reserve forests of Idagundi, Yallapura, of Uttara Kannada district of Karnataka, India. Samples of *M. fatua var. magnifica* were collected from the Katthalekan forests (14° 15' 50" N latitude and 74° 45' 35" E longitude) of Uttara Kannada district of Karnataka, India **(Fig.1a.& 1b.).** Fruit rind, aril, kernel and testa were separated. All the plant parts were cleaned with tap water and the parts without any damage or infection were selected for the study.

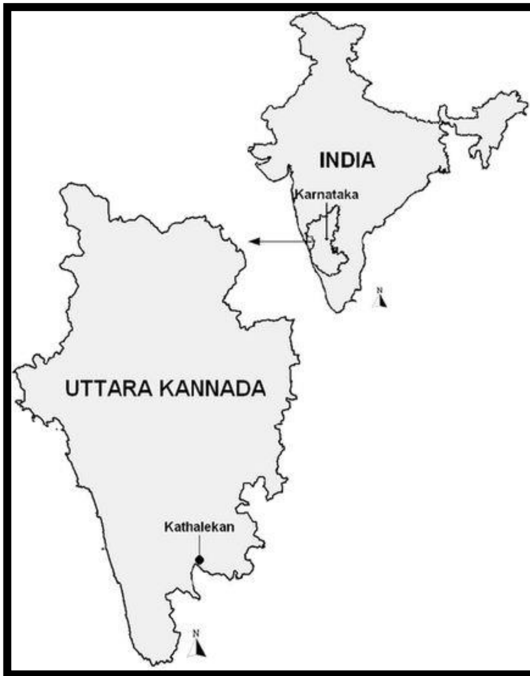


Fig. 1a.

Fig. 1b.

Physical attributes

Weight of whole fruit, seed, kernel and testa of randomly selected 50 seeds were determined (n=3). Length of fruit, seed length, width, thickness of testa etc. randomly selected 25 seeds were recorded using Vernier Callipers.

Estimation of Ash

Five gram of the sample was weighed in a silica crucible and heated in a muffle furnace for about 5-6 hours at 500℃, cooled and weighed (Indrayan et al, 2005).

$$\text{Ash}(\%) = \frac{A - B}{C} \times 100$$

Where, A- Weight of crucible+ ash,
B- Weight of crucible, C-weight of sample.

Moisture content

Moisture content of the samples was determined after drying at 100℃ to attain a constant weight (AOAC, 1990).

$$\text{Moisture content } (\%) = \frac{A - B}{A} \times 100$$

Where, A- Weight of the sample before drying (g),
B- Weight of the sample after drying (g).

Estimation of Crude Protein

Hundred milligram of sample was digested with 5ml of 36N H_2SO_4 after adding digestion mixture (K_2SO_4, CuSO4, Selenium:: 20:1:1) for 2-3 hours at 360° C. The digested samples were used to determine total nitrogen employing Micro Kjeldahl (Jackson, 1976) method. The protein content was calculated by multiplying the estimated nitrogen by 6.25(AOAC, 1990).

$$\text{Nitrogen}(\%) = \frac{(S - B) \times N \times 0.014 \times D}{W \times V} \times 100$$

Where S- Titration reading of sample, B- Titration reading of blank, N- Normality of HCL, D- Dilution Factor, W- Weight of Sample taken V- Volume of sample taken for distillation, 0.014- Milli equivalent weight of nitrogen.

Estimation of fat

Two grams of moisture free sample was extracted with n-hexane and extract was concentrated. The difference in the weight of evaporating flask and the weight of evaporating flask with extract was recorded as the weight of crude fat and expressed in percentage (Sreenivasan, 1968).

$$\text{Crude fat (\%)} = \frac{B-A}{C} \times 100$$

Where A-Weight of evaporating flask (g)

B- Weight of evaporating flak + fat (g)

C- Weight of sample taken (g).

Estimation of Fibre

Two gram of moisture free and fat free material of each sample was treated with 200ml of 1.25% H_2SO_4. After filtration and washing, the residue was treated 1.25%NaOH. It was filtered and washed with hot water, 1%HNO_3 and again with hot water. The washed residue was dried in an oven at 130℃ to constant weight and cooled in desiccators. The residue was transferred to a pre-weighed crucible weighed and ashed at 550° C for two hours, cooled and reweighed. Crude fibre content was expressed as percentage loss in weight on ignition (AOAC, 1990).

$$\text{Fibre(\%)} = \frac{A - B}{A} \times 100$$

Where, A-Weight of crucible with dry residue (g)

B-Weight of crucible with Ash (g),

A- Weight of Sample taken (g).

Estimation of Total Carbohydrate

Percentage of total crude carbohydrate was given by 100-(crude protein +crude lipid+ crude fibre+ ash) (AOAC, 1990).

Gross Energy Value

Gross Energy Value (kcal) of each sample were determined by multiplying the percentage of crude protein, fat and carbohydrate by the factors of four, nine and four respectively and adding up the values (Dir.90/496/CEE).

Estimation of Protein

Five hundred milligrams of sample was extracted with phosphate buffer (pH 7.0) and used for the estimation of protein by using method of Lowry et al.(1951).100µl of extract was mixed with 5 ml of alkaline copper tartarate reagent and incubate it for 10 min. It was added with 0.5 ml of FC reagent and incubated at room temperature for 10 min. Then blue colour developed was read at 660nm.BSA was used as standard.

Estimation of Starch and sugars:

500 mg of the seed sample was extracted with 20mL of the 80% ethanol and centrifuged at 2500 rpm for 10 min. The supernatant was used for sugar estimation and the residue was used for starch estimation.

Sugar was estimated by following Phenol Sulphuric acid method (Dubois et al, 1956). To the One mL of the supernatant 1mL of 18% redistilled phenol in water, followed by the rapid addition of 5mL of 96% H_2SO_4. After cooling optical density of the reaction mixture recorded at 490nm using spectrophotometer.

Starch was estimated by using Anthrone reagent following Hodge and Hofreiter, (1962) method. The residue was dried in hot air oven at 80C for 24 hours and digested with 52% perchloric acid for 2 hour at room temperature and centrifuged at 6000 rpm for 10 min. The supernatant was made up to 100mL with water. To 0.1mL of the aliquots 0.9ml of water and 4mL of Anthrone reagent were added and reaction mixture was kept in boiling water bath for 8mn. The test tube containing reaction mixture was cooled rapidly and optical density of the green colour was measured at 630nm.

Estimation of reducing sugar

Reducing sugar was estimated by Dinitrosalicylic acid method (Miller, G.L., 1972). Hundred milligram of the sample was weighed and sugars were extracted with 10 ml of hot 80% ethanol twice. Supernatant was collected and evaporated it in water bath at 80° C. About 10mL of distilled water was added sugars were dissolved. About 0.5mL of the extract was taken, made up to 3mL with distilled water and shaken well. After adding 3mL of DNS reagent, the reaction mixture was heated in a boiling water bath for 5 min. When the reaction mixture is still warm, 1mL of 40% Rochelle salt solution was added and cooled. Optical density the red colour of the reaction mixture

was measured at 510nm. Glucose was used as standard and the amount of the reducing sugars present in the sample was calculated using the standard graph plotted.

Estimation of Vitamin A

One gram of the sample was grinded to a paste with 1ml of the saponification mixture (2N KOH in 90% alcohol) and refluxed gently at 60̊ C for about 20 minutes. After cooling the reaction mixture to the room temperature, 20 ml of the water was added and mixed well. Vitamin A was extracted with 10ml portions of petroleum ether in a separating funnel thrice and the extract was pooled. Anhydrous sodium sulphate was used to remove moisture for 30-60 minutes. Five ml aliquot of the extract was dried at 60̊ C and dissolve the residue in 1ml of chloroform. Standard Vitamin A acetate was pipetted out in the range of 1.5-7.5µg in to series of clean test tubes and made up to 1ml using chloroform. 2ml of the T.C.A. was added and rapidly mixed. Absorbance was measured at 620nm immediately. In the same manner absorbance of the test samples were also performed. Standard graph was constructed and calculated the amount of Vitamin A/g tissue of the sample.

Vitamin- C

Hundred milligram of the sample was extracted with 4ml of 5% Tri Chloro Acetic acid using mortar and pestle. Homogenate was filtered and used as test sample. 0.2, 0.4, 0.6, 0.8, 1.0 ml of standard was pipette out into a series of test tubes and 500µl of the different test samples were taken made up to 1 ml with 5% T.C.A. After adding one ml of 2% DNPH reagent all the reaction mixture was kept in boiling water bath for 10 min (orange colour appears) and cooled. After adding 4ml of 65% of H_2SO_4, shaken well and incubated at room temperature for 30 min. Optical density was determined at 540nm using spectrophotometer.
Ascorbic acid was used as standard.

Estimation of Minerals:

Estimation of minerals like Calcium, Copper, Iron, Lead, Potassium, Sodium, and Zinc were performed according to Tandon (1993). Hundred milligram of moisture free sample (100mg) was digested with di–acid mixture (HNO_3:$HClO_4$: 5:1) for 2 hours at 220c. After cooling ,the mixture volume was made up to 25 ml with distilled water

and the digest was used for the estimation of minerals by Atomic absorption spectroscopy(Model: GBC 932 Plus; Australia).

For the estimation of calcium, potassium and sodium flame emission spectroscopy (Flame photometer 128, Systronics) was used.

Amino acid profiling:

The amino acid content of various samples was assessed based on Hofmann et al, (2003, 1997). Known amount of seed flour were hydrolyzed with Hcl (6N, 15ml) for 4 hours (at 145˚ C). For tryptophan, alkaline extraction and for sulphur containing amino acids, oxidised samples were used. After cooling, HCl was eliminated using rota evapourator (Biichi, Laboratorium Stechnik AG RE 121; Switzerland) with a diaphragm vaccum pump (MC2C; Vaccubrand GmbH, Germany). Trans-4(Aminomethyl) - cyclohexaneecarboxylic acid (Aldrich, purity 97%) as internal standard was added to each sample. The derivatization consisted of esterification with triflouroacetylation (Brand et al, 1994). The standard amino acids were weighed in reaction vials and dried (CH_2Cl_2) under a gentle stream of helium with slow heating in an oil bath (40-60˚ C) to remove traces of water. About 12 ml of fresh acidified isopropanol (acetyl chloride, 3ml+2- propanol, 12 ml) was added and the mixture was heated (100˚ C, 1 Hour). On cooling, the reagent was removed by a gentle stream of helium (60˚ C). To remove propanol and water, evaporation with three successive aliquots of CH_2Cl_2 was followed. The dry residue was triflouracetylated with triflouroacetic anhydride (200ml) for overnight at room temp. An aliquot of this solution was used without any treatment for Gas Chromatography –Combustion-Isotope ratio Mass Spectrometry (GC-C-IRMS/MS). The measurements of GC-C-IRMS/MS were carried out using gas chromatograph (Hewlett Packard 58590 II; Germany) connected through a split with a combustion interface to the IRMS system (GC –C II to MAT 252, Finnigam MAT; Germany) for isotopic determination of nitrogen and through a transfer line with a mass spectrophotometer (GCQ, Finnigam MAT; Germany) for qualitative and quantitative analysis of amino acids. The capillary column of GC (50m X 0.32mm i.d.X 0.5µm BPX5, SGE), operating with the carrier gas flow (1.5ml/ min.)with following temperature and pressure initial 50̊ C (1min.) increased to 100̊ C at 10̊ C/min (10min.) increased to175˚ C at 3˚ C/min (10 min.) and increased to 250˚ C/min (10min.)(Head pressure 13 psi, 90kpa).

Fatty acid profile:

The GC-MS analysis was done by electron impact ionization (EI) method on Auto system XL gas chromatography (Perkin Elmer Instrument, Germany) coupled to a Turbo mass spectrophotometer (Perkin Elmer Instrument, Germany). The column was fused silica capillary column, mμ30 × 0.25 mm ID; coated with D-I, 0.25 film thickness. The temperature of column was programmed at 70 to 250 °C at the rate of 10 °C /min increase, injection port temperature at 250 °C. Helium was used as carrier gas at constant pressure of 100 kpa and flow rate of 20 ml/min. Samples which dissolved in chloroform was run fully at range of 60-550 amu and the results were compared by using NIST 107 Spectral library search programme.

Essentail Oil Extraction:

About 500gm of the leaves were cut into medium sized pieces and were hydro distilled using Clevenger type distillation apparatus5. Distillation process was done for 24 hours and the oil was extracted. The oil obtained was refrigerated at 5°C till further use.

Estimation of Phenolics:

One gram of the seed sample was homogenised and extracted with 10 times volume of 80% ethanol and centrifuged at 10,000 r .p. m. for 20 min. Supernatant was used for the estimation of phenolics. Supernatant was dried in water bath. To the precipitate 2ml of distilled water was added and mixed properly. From that 250µl of the sample was pipetted in to test tube and at the same time Standard Gallic acid was pipetted out into a clean test tube. Using distilled water it was made up to 1ml. 2ml of 2% sodium carbonate followed by 100µl of FC reagent were added to both test and standard test tubes and kept for half hour incubation at dark. The blue coloured developed was read using spectrophotometer at 650nm. A standard curve was prepared using different concentrations of Gallic acid.

From the standard curve, the concentration of phenols in test samples was found out and expressed in mg phenols/ 100 g material.

Estimation of Tannin (Schanderl, 1970)

Accurately Weighed 0.5g of the powdered material was transferred to a 250mL conical flask. Add 75mL water. Heat the flask gently and boil for 30 min. Centrifuged at 2,000rpm for 20 min and collect the supernatant in 100mL volumetric flask and make up the volume. Transfer 1mL of the sample extract to a 100mL volumetric flask containing 75mL water. Add 5mL of Folin-Denis reagent, 10mL of sodium carbonate solution and dilute to 100mL with water and Shaken well. Read the absorbance at 700nm after 30 min.

Tannic acid was used as standard. The tannin concentration was determined by the standard graph of tannic acid solution.

Estimation of Saponin

Twenty grams of powdered sample was used for estimation of saponins by Obadoni & Ochuko (2001) method. Each sample is added with 100ml of 20% ethanol and mixed properly with shaker for 30 minutes. After heating at 55°C in water bath for 4 hour, it was filtered and extracted the residue with 200ml of 20% aqueous ethanol. After combining the extracts, volume is reduced to 40ml in water at 90°C and extract twice with 20ml of diethyl ether in a separating funnel. To the collected aqueous layer 60ml of n-butanol is added and butanol layer is collected. It was washed twice with 10ml of 5% Nacl to remove impurities. Remaining solution was heated in water bath till evaporation and dried in hot air oven at 40°C.

Saponin content was expressed as percentage of the initial sample.

Extraction

The dried plant parts like leaf (25g), bark (25g), aril (25g), kernel (25g),testa (25g), fruit rind (25g) were powdered using a mortar and pestle. These plant samples were extracted with different polar and nonpolar solvents like methanol, ethyl acetate, chloroform, hexane and petroleum ether by Soxhlet extraction method. Aqueous extract is done by water bath method. The extracts were concentrated and stored at 4°C until use.

Yield of extract

The extracts obtained with each solvent were weighed and the yield was expressed in percentage.

$$\text{Yield}(\%) = \frac{\text{Weight of residue}}{\text{Weight of sample taken}} \times 100$$

Phytochemical Analysis

Preliminary phytochemical screening of solvent extracts was carried out by following Harborne (1992). Extracts were dissolved in their respective solvent (100mg/ml) and used in different estimation.

1. Test for Alkaloids

Hager's test: One ml of the plant extract was added with few drops of Hagers's reagent formation of the yellow precipitate will indicate the presence of alkaloids.

Wagner's test: One ml of the palnt extract acidified with 1.5% Hcl and added with few drops of Wagner's reagent, formation of yellow precipitate will indicate the presence of alkaloids.

Mayer's test: Formation of yellow or white precipitate on the addition of few drops of Wagner's reagent to one ml of plant extract will indicate the presence of alkaloids.

2. **Test for Flavonoids**: A test tube containing 0.5ml of extract is added with 10% H_2SO_4, cooled and few drops of chloroform were added brown precipitate indicated the presence of flavonoids.

3. **Test for Saponin**: Formation of the honeycomb like froth when the plant extract dissolved in water and shaken vigorously is the sign of presence of saponins.

4. **Test for tannins**: Solution of the extract is mixed with few drops of 1% lead acetate gives black precipitate indicates the presence of tannin.

5. **Test for steroids**: A small amount of dried extract is dissolved in 2ml of chloroform and sulphuric acid is added along the side of the test tube. Formation of reddish brown ring will indicate the presence of steroids.

6. **Test for glycosides:** To 2ml of the extract solution two drops of 10% α-napthol and mixed and added with 2ml of conc. Sulphuric acid along the sides of the test tube.

7. **Test for Phenols**: To one ml of the sample solution 2ml of the distilled water followed by the addition of few drops of 10% aqueous ferric chloride solution.

8. **Test for Resins**: Dissolving the extract in acetone and upon pouring the solution into distilled water formation of turbidity will indicates the presence of resins.

Determination of Total Phenolic content

The total phenolic content was measured using the Folin- Ciocalteau method (Taga et al,1984)Hundred µl of the extract (10mg/ml) was mixed with 2ml of 2% Na_2CO_3 and allowed to stand for 2 min at room temperature, followed by the addition of 100µl of 50% Folin- Ciocalteau's phenol reagent. After incubation for 30 min at room temperature in darkness, the absorbance was read at 720nm using spectrophotometer (Systronics-166). The total phenolic contents of the samples were expressed as mg Gallic acid equivalent per gram of the extract (mg GAE/g).

Determination of Flavonoid content

Total flavonoid content was determined following the Aluminium Chloride method (Zichen et al, 1999). A known volume of each of the extract was made up to 4ml using distilled water followed by the addition of 0.3ml of $NaNO_2$ (1:20). After 5 min, 0.3ml of 10% $AlCl_3.H_2O$ solution was added. After the 6th min, 2ml of 1M NaOH solution was added and the total volume was made up to 9ml using distilled water. The absorbance against blank was determined at 510nm. Results were expressed as mg Quercetin equivalents (QE)/g of extract.

***In vitro* Antioxidant Assay**

The antioxidant activity of plant extracts was determined by *in vitro* method the DPPH (2, 2- Diphenyl -1-picryl hydrazyl) free radical scavenging activity and reducing power assay.

Evaluation of DPPH scavenging activity (Liyana and Shahidi, 2005)

A solution of DPPH (0.135mM) in methanol was prepared and 1ml of this solution was mixed with 1ml of varying concentrations of the extracts. The reaction mixture was vortexed thoroughly and left in dark at room temperature for 30min. The absorbance of the mixture was measured at 517nm using Ascorbic acid as standard. The ability to scavenge DPPH free radical was calculated as

$$\%\text{DPPH radical scavenging activty} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample}}{(\text{Absorbance of control}} \times 100$$

Reducing Power Assay (Oyaizu, 1986)

Hundred µl of the extracts of varied concentrations were mixed with phosphate buffer (2.5ml, 0.2 M, P^H 6.6) and 1% potassium ferricyanide (2.5ml). The mixture was

incubated at 50˚C for 20 min. 2.5ml of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000 rpm for 10 min. 2.5ml of the supernatant was mixed with 2.5ml of distilled water and freshly prepared $FeCl_3$ solution (0.5ml, 0.1%). The absorbance was measured at 700nm. Reducing power was expressed as ascorbic acid equivalent (AAE) in milligram per gram of the extract.

Antimicrobial activity by disc diffusion method

Five bacterial cultures viz. Two Gram-positive (*Bacillus subtilis,* ATCC 6633 *and Staphylococcus aureus,* NCIM 2079) and three Gram-negative (*Proteus vulgaris,* NCIM 2813*, Pseudomonas aeruginosa,* NCIM 2200 and *Escherichia –coli,* NCIM 2931) were obtained from National Chemical Laboratory, Pune , India and were maintained on nutrient agar slants. Two hundred µl of the overnight grown culture of each organism was dispensed into 20ml of sterile nutrient broth and incubated for 4-5 h at 37˚C to standardise the culture to 10^{-5} CFU/ml.

Two fungal strains viz. *Aspergillus niger* MTCC No. 1344 and *Candida albicans* MTCC No. 227 were obtained from IMTECH, Chandigarh, India. The fungal cultures were maintained on potato dextrose agar (PDA) were sub cultured and incubated for 6 days. The spores from the fungal cultures were suspended in sterile distilled water and the same was used for antifungal studies.

Antibacterial activity assay was carried out by disc diffusion method. For this 0.1ml (10^{-5} CFU/ml) of 24 h old bacterial culture was placed on Muller Hinton Agar medium and spread throughout the plate by spread plate technique. Sterile paper discs (5mm in diameter) obtained from Himedia, impregnated with 25µl of the extract (10mg/ml) was placed on the surface of the medium and incubated at 37˚C for 24 h. Antibacterial activity was recorded by measuring the diameter of zone of inhibition. Tetracycline was used as positive reference standard. The antifungal study was carried out by inoculating the fungal spores on PDA medium pre impregnated discs with 25µl of plant extracts. Nystatin was used as a positive reference standard. The experiment was performed in triplicate.

Determination of Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)

MIC of the DMSO extracts was determined by using different concentrations of extracts in Mueller-Hinton broth for bacteria by macro dilution method. [15] The

lowest concentration of the DMSO extract inhibiting the visible growth of microorganisms was considered as MIC. The MBC was determined by sub culturing the test dilution on to a fresh drug-free solid medium and incubated for 18–24 h. The highest dilution that yielded no single bacterial colony on a solid medium was taken as MBC.

In-vitro studies for determination of α-amylase and α-glucosidase activity (modified Kim et al, 2000)

To 50µl of enzyme alpha amylase (obtained from Sigma), 2µl of plant extract was added and made up to 70µl using 50Mm phosphate buffer of p^H 5.8 (which contained 2g/L of BSA and 0.2g/L NaN_3) in 96 well micro plate, shaken well, optical density was measured at 405nm using Synergy H1 micro plate (Model no.1404039) reader and kept for incubation at room temperature for 5minutes. About 50µl of substrate was added (1% starch) and incubated for 5 minutes at room temperature and optical density was measured at 405 nm. Percentage Inhibition was measured by using the formula,

% Inhibition= 100-[(Final optical density-Initial optical density) X 100].

To 40µl of enzyme alpha glucosidase (obtained from SRL, India), 2µl of plant extract was added and made up to 60µl using 50Mm phosphate buffer (p^H 5.8) in 96 well micro plate and kept for incubation at 37°C for 10 minutes. To this, 40µl of substrate, p-Nitrophenyl alpha D-glucopyranoside was added. After10 minutes of incubation at 37°C, 70µl of Na_2CO_3 were added and optical density wasvmeasured at 410nm. Acarbose (obtained from Sigma) was used as standard in both the estimation. Wells without standard or test sample considered as positive control while prior addition of Na_2CO_3 served as negative control. Parentage inhibition was measured by using the formula as,

$$\%\text{Inhibition} = 1 - \frac{(\text{Absorbance of test} - \text{Absorbance of negative control})}{(\text{Absorbance of positive control})} \times 100$$

In-vitro studies for determination of anti- inflammatory activity (Padmanabhan P and Jangle NS, 2012)

The blood was collected from a healthy human volunteer and collected in a heparinised centrifuge tube washed thrice with 0.9% saline solution and centrifuged simultaneously for 10 minutes at 3000 rpm. The residue contained RBC and a 40% v/v

suspension made by using isotonic phosphate buffer at p^H 7.4 was used as RBC suspension. 30µl of the RBC suspension was mixed with 1ml of isotonic phosphate buffer and 2ml of 0.35% hypo saline to which 10µl of the plant extract was added and kept for incubation at room temperature for 10minutes.Centrifugation was done for 10 minutes at 3,000rpm and the supernatant was taken to read the optical density at 540nm.

Percentage haemolysis was calculated by using the formula:

$$\%\text{Haemolysis} = \frac{\text{Optical Density of test}}{\text{Optical density of control}} \times 100$$

Percentage inhibition was calculated using formula,

$$\%\text{Inhibition} = 100 - \text{Percentage of haemolysis}$$

In-vivo Pharmacological studies

Acute oral toxicity study

The acute oral toxicity of ethyl acetate extract of *M. fatua var. magnifica* kernel and aril was carried out in adult female albino rats weighing about 150-200 g, by up and down method as per OECD 425 guidelines (OECD, 2001). The test procedure minimizes the number of animals required to estimate the oral acute toxicity. The test also allows the observation of signs of toxicity and can also be used to identify the chemicals that are likely to have low toxicity. Animals will be fasted (food but not water withheld overnight) prior to dosing. The fasted body weight of each animal will be determined, and the dose will be calculated according to the body weight (Ghosh, 2005).

Analgesic activity

1. Eddy's Hot plate method:

Overnight fasted mice should be divided into 5 groups (5 animals in each group) as shown bellow.

- **Group 1:** 1 % Tween 20 (10ml/kg, *p. o.*)
- **Group 2:** Single dose of Pentazocine (10mg/ kg body weight, *p. o.*)
- **Group 3:** Single dose of extract (100mg/kg body weight, *p. o.*)
- **Group 4:** Single dose of extract (200mg/ kg body weight, *p. o.*)
- **Group 5:** Single dose of extract (400mg/kg body weight, *p. o.*)

All the treatments were given 30 min prior to placement of the mice on hot plate. The basal reaction time should be taken by observing hind paw licking or jump response (whichever appear first) in animals while placed on hot plate, which should be maintained at constant temperature 55 ± 0.5°C. A cut-off period of 10 sec should establish to prevent damage to the paws (Franzotti et al, 2000). Reaction time (sec) should reinvestigate at 30, 60, 90 and 120 min after oral administration of the drug solutions.

2. Tail immersion test

The tail immersion method used to evaluate the central mechanism of analgesic activity (Upudha et al, 2007). Overnight fasted Wister rats should divide into 5 groups (5 animals in each group).

- **Group 1:** Aqueous suspension of 1% Tween-20 (10ml/kg, *p. o.*)
- **Group 2:** Pentazocine (10mg/kg) dissolved in 0.9% saline (*p. o.*).
- **Group 3:** Single dose of extract (100mg/kg body weight, *p. o.*)
- **Group 4:** Single dose of extract (200mg/kg body weight, *p. o.*)
- **Group 5:** Single dose of extract (400mg/kg body weight, *p. o.*)

All the treatments should be given 30 min. prior to test. After administration of doses two cm of the tail of rat should immerse in warm water kept constant at 55± 0.5°C. Time taken by the rats to deflect their tails should take as reaction time or tail flick latency (sec). A latency (delay in tail flick) period of 10 sec is defined as complete analgesia and the measurement was then stopped to avoid injury to mice. The latent period of the tail-flick response determined at 30, 60, 90 and 120 min after the administration of drugs. Basal reaction should be taken before the administration of drug.

Anti inflammatory activity

The dose which showing higher analgesic activity should select for this activity. The carrageenan-induced paw edema assay (Winter et al, 1962). Overnight fasted adult rats should divide into 4 groups (4 animals in each group).

- **Group 1:** Aqueous suspension of 1% Tween-20 (10ml/kg body weight., *p. o.*)
- **Group 2:** Single dose Diclofenac in 0.9% saline (10mg/kg body weight, *p. o.*).
- **Group 3:** Single dose of extract (200mg/kg body weight, *p. o.*)
- **Group 4:** Single dose of extract (400mg/kg body weight, *p. o.*)

All the treatments given 30 min prior to edema induction. Edema should induce on the right hind paw of rat by subplantar injection of 0.1 ml solution of 1% carrageenan sodium [in 0.9% NaCl (w/v)]. The swelling of the carrageenan-induced feet should measure using plethysmometer (Almemo 2290-5, IITC Life Sciences, Wood land Hills, USA) before the injection of carrageenan (zero time) and at 0.5, 1, 2, and 3h after the injection of carrageenan. Percentage edema inhibition should calculate according to Gupta et al. (2006) and Sawadogo et al. (2006) as

$$\%\text{Inhibition} = \frac{\text{D0} - \text{Dt}}{\text{D0}} \times 100$$

Where D_0 was Mean paw diameter of control group at a given time and

D_t was paw diameter of treated (extract or standard) group at the same time.

Anti hyperlipidemic activity

Hyperlipidemia should induce in Wistar albino rats by single intraperitoneal (i.p.) injection of freshly prepared solution of Triton-X-100 (100 mg/kg) in physiological saline solution (0.9%) after fasting for 18 h. The rats should be divided into five groups of five animals each.

- Group 1 normal control rats, which receiving 1% Tween 20 (10 ml/kg body weight) (p.o).
- Group 2 hyperlipidemic group which receiving Triton-X-100 (100 mg/kg) (i.p) in normal saline [0.9% NaCl (w/v)].
- Group 3 test group receiving Triton-X-100 (100 mg/kg body weight) (i.p) in normal saline + Plant extract (400 mg/kg) (p.o).
- Group 4 test group receiving Triton-X-100 (100 mg/kg body weight) (i.p) in normal saline + Plant extract (400 mg/kg) (p.o).
- Group 5 standard group receiving Triton-X-100 (100 mg/kg body weight) (i.p) in normal saline + Atorvastatin (10mg/ml) (p.o).

All the treatment should be given immediately after injection of Triton-X-100, i.p., except in control groups. In the following period of study (24 h), animals should have an access only to water. After 24 h of treatments, animals should mildly anaesthetize with diethyl ether and blood was withdrawn from the plexus of eye vein using heparinised capillary tube. The serum should be assayed for total cholesterol

(TC), triglycerides (TG), high-density lipoprotein (HDL) using standard kits provided by AGAPE diagnostics, Mumbai. Low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) and atherogenic index (AI) was calculated by Friedwald formula (1972).

Anti diabetic activity: Streptozotocin induced model in Wistar albino rats (Biswas et al, 2011).

Diabetes Induction

Diabetes mellitus was induced in overnight fasted rats by a single intra peritoneal injection of Streptozotocin (50mg/kg body weight). After 3 days, fasting blood glucose levels were measured and the animals showing blood glucose level of 225 mg/dl were used for the study.

The rats should be divided in to five groups (n=6) as bellow.

- **Group 1:** Normal nondiabetic control rats
- **Group 2:** Diabetic control rats – STZ induced.
- **Group 3:** Diabetic rats+ Single dose of Glibenclamide (0.5mg/kg b.w., *p.o.*)
- **Group 4:** Diabetic rats+ Single dose of extract (200mg/kg kg b.w., p.o)
- **Group 5:** Diabetic rats+ Single dose of extract (400mg/kg kg b.w., p.o)

Group 1 were served as normal non diabetic group of rats while remaining other groups were group of diabetic rats. Group 2 served as (STZ induced) diabetic control group of rats. Group 3 served as standard drug Glibenclamide (0.5mg/kg body weight, p. o.). Group 4 and 5 were administered with ethyl acetate extract of kernel at the concentrations of 200 and 400mg/kg body weight (p. o.) respectively. Both the standard drug as well as plant extracts were administered daily for 15 days. Using Transasia Biomedicals Limited Biochemistry Analyzer (CHEM 5X, Mumbai) fasting blood glucose level of the rats were measured by following Trinder’s method (1969) at the interval of 5 days.

Isolation of compounds

Chromatographic Purification

Thin Layer Chromatography was carried out to isolate the principle components that were present in most effective seed and aril extracts of *M. fatua*. Thin Layer Chromatography studies were carried out for different extracts on Silica gel (G) 60 F. The different solvent systems of different polarities were prepared and TLC

studies were carried out to select the solvent system capable of showing better resolution.

Solvent phase

The different solvent systems were used for different extracts. For hexane extract of *M. fatua* kernel Benzene: Ethyl acetate: Hexane (4:1:1) and Toluene: Hexane: Ethyl acetate (8:2:4) were used. Toluene: Ethyl acetate: (10:1) was used for thin layer chromatography of *M. fatua* aril ethyl acetate extract. For chloroform extract of *M. fatua* aril Hexane: Ethyl acetate (1:1) as well as Toluene: Ethyl acetate (20:1) were the mobile phase.

Method

The above prepared plant extracts were applied on pre-coated TLC plates by using capillary tubes and developed in a TLC chamber using suitable mobile phase. The developed TLC plates were air dried and observed under ultra violet light UV at both 254 nm and 366 nm. They were later sprayed with different spraying reagents and some were placed in hot air oven for 1 min for the development of color in separated bands. The movement of the analyte was expressed by its retention factor (R_f). Values were calculated for different sample.

$$\mathrm{Rf} = \frac{\text{Solute front}}{\text{Solvent front}}$$

Attenuated Total Reflector:

Attenuated total reflection (ATR) is a sampling technique used with infrared spectroscopy which enables samples to be examined directly in the solid or liquid state without further preparation.

Different fractions of the ethyl acetate extract of *M. fatua* were placed over the attenuated total reflector (IR, Prestige-21, Shimadzu) with a scan range from 400- 4000 cm^{-1} with a resolution of 4 cm^{-1} using Hap Zingel method and analysed. No. of scans given was 20. Using IRsolution software the possible functional groups in the fractions were determined.

RESULTS

Table 1. showing some physical attributes of *M. fatua* and *M. dactyloides*.

Physical Attributes	*M. fatua*	*M. dactyloides*
Weight of fresh Fruit(g)[a]	60.33±0.4	55.67±0.72
Weight of rind (g)[a]	37.91±0.2	35.24±0.2
Weight of fresh Aril(g) [a]	1.96±0.6	1.45±0.2
Weight of fresh Seed(g)[a]	20.46±0.2	18.98±0.13
Fruit length(cm) [b]	5.6±0.07	4.428±0.63
Fruit diameter(cm)[b]	4.25±0.5	4.43±0.3
Seed length(cm)[b]	4.23±0.31	3.404±0.62
Seed thickness(cm) [b]	1.93±0.1	2.43±0.45
Kernel thickness(cm) [b]	1.8±0.23	2.24±0.7
Testa thickness(cm)[b]	0.13±0.1	0.19±0.1

a- mean of 100 fruits/ seeds
b- mean of 25 fruits/ seeds

Physical attributes of *M. fatua* and *M. dactyloides* fruits were represented in **Table 1.** *M. fatua* fruit and parts are comparatively larger than the fruit and fruit parts of *M. dactyloides* except in thickness. *M. fatua* showed a rind: aril: seed ratio of about 62.83:3.24:33.91 while *M. dactyloides* showed 63.30:2.60:34 ratio.

The morphological features of the both the plants were shown in **fig. 2 & 3.**

Fig.2a.

Fig. 2b.

Fig.2a-b. Views of Myristica swamp

Fig. 2c. *M. fatua* tree trunk

Fig. 2d. Knee root of *M. fatua*

Fig. 2e. ***M. fatua*** **Leaf**

Fig. 2f. Inflorescence bearing clusters of flower and flower buds

Fig. 2g. Branch bearing *M. fatua* fruits (immature)

Fig. 2h. *Myristica fatua* fruit (Opened)

Fig. 2i. Arillated seed of *Myristica fatua*

Fig. 2j. Seed of Myristica fatua var. magnifica

Fig.3a. Leaf of *M. dactyloides*

Fig.3b. Fruit of *Myristica dactyloides*

Fig. 3c. Fruit (Opened)

Fig. 3d. Yellowish aril of *M.dactyloides*

Fig.3e. Seeds of *M. dactyloides*

Table2. Proximate studies *on M. fatua var. magnifica.*

Composition	Kernel	Testa	Aril	Rind	F value
Ash	2.12±0.07[c]	5.8±0.50[b]	3.02±0.12[c]	14.27±0.24[a]	744.04
Moisture	35.67±0.61[c]	21.2±1.98[d]	48.67±0.94[b]	75.57±0.41[a]	824.58
Crude Nitrogen	1.62±0.07[a]	0.588±0[b]	0.588±0.13[b]	0.308±0.03[b]	0.997
Crude Protein	10.15±0.49[a]	3.68±0[b]	3.68±0.85[b]	1.92±1[c]	187.16
Crude fat	17.37±0.15[a]	4.03±0.15[c]	13.51±0.12[b]	0.767±0.05[d]	11108.77
Crude fibre	6.67±0.15[a]	5.2±0.1[b]	5.13±0.25[b]	2.33±0.05[c]	393.42
Crude carbohydrate	36.30±0.78[b]	18.70±0.63[d]	25.35±0.80[c]	80.71±0.54[a]	4673.58
Energy	343.43±3.3[a]	126.17±3.17[c]	237.71±3.92[b]	337.43±1.2[a]	3271.07

Table2. Indicates the significantly higher ash content of 14.27% in fruit rind followed by testa (5.8%) of *Myristica fatua var. magnifica*. Rind recorded significantly high moisture content (75.57%) while testa recorded least moisture content (21.2%). Crude protein was found to be higher in Kernel (10.15%) followed by on par protein (3.68%) was recorded in testa as well as aril. Significantly higher amount of lipid content (17.37%) was registered in kernel followed by aril (13.5%) while lipid content was comparatively low in rind (0.76%). About 6.67% of fibre content was recorded in kernel while testa and aril showed on par fibre content. The order of the carbohydrate content was in the order of Rind (80.71) > Kernel (36.30%) >Aril (25.35%) > Testa (18.70%). Calorific value of the kernel (343.43%) was significantly higher compared to other fruit parts.

Table 3. Proximate studies *on M. dactyloides.*

Composition	Kernel	Testa	Aril	Rind	F value
Ash	3.45±0.04^{b}	1.6±0.29^{d}	2.61±0.02^{c}	11.67±0.33^{a}	834.47
Moisture	47.67±2.62^{b}	23.17±2.32^{d}	39.41±1^{c}	60.33±0.47^{a}	143.639
Crude Nitrogen	1.28±0.07^{a}	0.73±0.04^{b}	1.42±0^{a}	0.27±0.05^{c}	204.335
Crude Protein	8.05±0.49^{a}	4.58±0.29^{b}	8.93±0^{a}	1.67±0.29^{c}	242.346
Crude fat	16.17±0.25^{a}	4.63±0.15^{c}	10±0.1^{b}	0.44±0.05^{d}	338.801
Crude fibre	7±0^{a}	5.83±0.20^{b}	6.11±0.12^{b}	3.63±0.11^{c}	15218.617
Crude carbohydrate	34.53±0.63^{b}	13.91±0.16^{d}	27.67±0.10^{c}	82.59±0.51^{a}	1807.42
Energy	315.3±6^{b}	115.68±2^{d}	236.27±1.2^{c}	334.36±4.8^{a}	5591.344

Significantly a higher amount of ash (11.67%) was registered in rind of *M. dactyloides* **(Table 3.).** Rind recorded about 11.67% of ash content which was significantly high compared to kernel (3.45%), aril (2.61%) and testa (1.6%). Higher amount of moisture was noticed in rind (60.33%) while testa recorded least moisture content of 23.17%. On par higher crude nitrogen as well as protein content (8.93 & 8.05% was noticed in case of aril and kernel. Kernel recorded 16.6% of fat followed by aril registered 10%. The fibre content in the *M. dactyloides* fruit parts were in the order of kernel (7%) > aril (6.11%) > Testa (5.83%) >Rind (3.63%). Significantly a good amount of crude carbohydrate was recorded fruit rind (82.59%) followed by kernel (34.53%) while testa registered least carbohydrate content of 13.91%. A significantly higher calorific value was found in fruit rind (334.36) while testa exhibited low calorific value.

Table 4: Protein, Carbohydrates and vitamins of *M. fatua*

Components	Kernel	Testa	Aril	Rind	F value
Total Protein	302±0.86^{b}	7±0.4^{d}	89.06±0.70^{c}	464±0.2^{a}	358339.68
Total Sugar	8.12±0.07^{b}	1.10±0.1^{c}	28.8±0.52^{a}	1.2±0.2^{c}	6123.54
Reducing Sugar	5.03±0.15^{b}	0.65±0.09^{c}	16.73±0.64^{a}	0.84±0.05^{c}	1524.964
Starch	34.9±0.5^{a}	22.32±0.06^{b}	10.45±0.13^{c}	10.23±0.05^{c}	5047.963
Vitamin- A	1.44±0.03^{b}	0.62±0.01^{d}	3.63±0.04^{a}	0.82±0.01^{c}	7836.140
Vitamin- C	64±0.2^{a}	10±0.2^{b}	41.3±0.36^{c}	30.6±0.52^{d}	12551.752

Significantly a higher amount of total protein of 464mg/ g was recorded in *M. fatua* fruit rind **(Table 4.).** Total sugar was significantly higher in aril (28.8mg/g) followed by kernel 8.12mg/g while rind and testa showed on par total sugar of 1.2 and 1.1mg/g respectively. Aril was rich in reducing sugar (16.73mg/g) which was significantly different. Kernel of *M. fatua* registered 34.9mg/g of starch followed by testa (22.32mg/g) while aril and rind exhibited on par comparatively lesser starch content. Vitamin A ranged between 0.62-3.63mg/g among different fruit parts and were highly significant. Kernel exhibited higher Vitamin C of 64mg/g followed by aril (41.3mg/g), rind (30.6mg/g) and testa (10mg/g).

Table 5: Protein, Carbohydrates and vitamins of *M. dactyloides.*

Components	Kernel	Testa	Aril	Rind	F value
Total Protein	254±0.2^{b}	14±0.8^{d}	101.6±0.52^{c}	379±0.2^{a}	314300.28
Total Sugar	3.4±0.34^{b}	0.19±0.03^{c}	31±1.11^{a}	0.87±0.01^{c}	1936.24
Reducing Sugar	2.38±0.02^{b}	0.99±0.10^{c}	22.53±0.50^{a}	0.67±0.03^{c}	5102.773
Starch	48.76±0.51^{a}	28.16±0.37^{b}	12.7±0.51^{d}	20.13±0.23^{c}	3978.135
Vitamin- A	6±0.2^{a}	0.83±0.02^{c}	1.64±0.04^{b}	0.4±0.02^{d}	1806.536
Vitamin- C	42±0.20^{c}	54±0.20^{a}	14.6±0.52^{d}	45.7±0.45^{b}	6134

Table 5., indicates that rind of *M. dactyloides* recorded significantly higher total protein of 379mg/g followed by kernel (254mg/g), aril (101mg/g) and testa showed 14mg/g of total protein. Significantly a higher total sugar was found in aril (31mg/g) while rind and testa exhibited least total sugar of 0.87 and 0.19mg/g. Reducing sugar was found in following order of Aril (22.53mg/g) > kernel (2.38mg/g) > testa(0.99mg/g) > rind (0.67mg/g) .Kernel exhibited a significantly higher starch of 48.76mg/g followed by testa (28.16mg/g) while aril recorded least of 12.7 mg/g of starch content. Vitamin A was found to be rich in kernel (6mg/g) while rind exhibited poor vitamin A content of 0.4mg/g.Rnd recorded significantly higher Vitamin C content of 54mg/g , followed by rind(45.75mg/g), kernel (42mg/g) and while aril showed comparatively least of 14.6mg/g of vitamin C.

Table 6.* Mineral composition of *Myristica fatua

Minerals	Kernel	Testa	Aril	Rind	F value
Calcium(Ca)	23.97±0.28[b]	24.47±0.50[b]	24.48±0.60[b]	26.7±0.65[a]	15.833
Chromium(Cr)	1.91±0.08[b]	3.25±0.27[a]	3.6±0.10[a]	0.62±0.04[c]	233.119
Copper (Cu)	0.68±0.02[a]	0.56±0.02[b]	0.68±0.01[a]	0.68±0.02[a]	27
Iron(Fe)	1.03±0.05[b]	0.54±0.02[c]	1.06±0.05[b]	2.3±0.1[a]	392.28
Nickel (Ni)	1.56±0.21[a]	1.58±0.01[a]	1.07±0.03[b]	0.66±0.05[c]	45.59
Potassium(K)	29.26±0.30[b]	37.67±0.57[a]	27.47±0.45[b]	23.8±1.05[c]	236.25
Sodium(Na)	37.4±0.52[a]	36.6±0.53[a]	36.86±0.80[a]	37.7±0.60[a]	1.8
Zinc(Zn)	1.42±0.04[b]	1.09±0.01[c]	1.68±0.01[a]	1.32±0.06[b]	104.87

Calcium, potassium and sodium were the major mineral elements recorded in the different parts of *M. fatua* fruit **(Table 6.)** Rind recorded significantly high amount of calcium (26.7mg/g) followed by aril (24.48mg/g), Testa (24.47mg/g) and kernel (23.97mg/g) exhibited on par calcium content. The order of the chromium content was aril (3.6mg/g) > testa (3.25mg/g) > kernel (1.91mg/g) > rind (0.62mg/g). On par copper content was registered by kernel, aril and rind. Fruit rind was significantly rich with iron (2.3mg/g) while testa showed low concentration of iron (0.54mg/g).Kernel and testa showed significantly higher nickel of about 1.58 and 1.56 mg/g respectively. Significantly a good amount of potassium was recorded in testa (37.67mg/g) while rind showed least (23.8mg/g). There was on par sodium content was observed in all the parts. Traces of zinc, ranging from 1.09- 1.42mg/g was recorded in different fruit parts of *M. fatua.*

Table 7:* Mineral composition of *M. dactyloides

Minerals	Kernel	Testa	Aril	Rind	F value
Calcium(Ca)	26.86±0.90[a]	24.56±0.85[b]	25.2±0.72[ab]	18.2±0.34[c]	79.53
Chromium(Cr)	2.87±0.30[b]	2.47±0.20[b]	4.47±0.02[a]	1.23±0.005[c]	78.159
Copper (Cu)	0.97±0.72[b]	1.18±0.30[a]	0.74±0.30[c]	0.20±0.20[d]	577.80
Iron(Fe)	0.51±0.01[b]	0.17±0.005[d]	0.76±0.03[a]	0.30±0.1[c]	69.24
Nickel (Ni)	1.067±0.05[b]	0.86±0.01[c]	1.39±0.011[a]	0.487±0.10[d]	122.81
Potassium(K)	21.93±0.40[b]	34.6±0.52[a]	19.8±0.20[c]	12.73±0.70[d]	1020.57
Sodium(Na)	37.53±0.92[a]	37.2±0.2[a]	37.67±0.57[a]	30.53±0.70[b]	97.754
Zinc(Zn)	1.8±0.1[a]	1.29±0.05[b]	1.28±0.07[b]	1.00±0.1[c]	47.106

Table 7. Indicates that Calcium, potassium and sodium were predominant mineral elements of *M. dactyloides*. Kernel recorded significantly high amount of calcium (26.86mg/g), followed by aril (25.2mg/g) while rind showed least amount of calcium of about 18.2mg/g. Chromium was significantly higher (4.47mg/g) in aril. Copper content was ranged between 0.2-1.18mg/g in different fruit parts. Iron content was comparatively low in all the parts compared to other elements. Nickel was found in the order of aril (1.39mg/g) > kernel (1.067mg/g) > testa (0.86mg/g) > rind (0.487mg/g). Significantly a higher concentration of potassium (34.6mg/g) was noticed in testa while rind showed least amount of potassium of about 30.53mg/g. On par sodium was recorded by kernel, testa and rind while rind showed 30.50mg/g of sodium. Kernel exhibited higher zinc of about 1.8mg/g while rind showed least of about 1mg/g of zinc.

Table 8. Amino acid profile of *M. fatua* fruits.

Sl. No.	Amino acid (%)	Kernel	Testa	Aril	Rind
1	Asp	8.18	7.29	6.17	5.68
2	Glu	9.77	6.45	6.04	7.58
3	Ser	5.11	6.16	5.96	5.88
4	Gly	9.74	10.50	11.2	12.27
5	His	2.57	2.68	0.53	0.48
6	Arg	5.44	3.74	1.87	4.28
7	Thr	3.62	3.93	0.91	3.92
8	Ala	7.18	7.11	6.53	7.72
9	Pro	7.94	8.76	8.84	9.92
10	NH3	3.14	4.90	7.89	4.95
11	Tyr	2.91	2.69	8.45	1.85
12	Val	7.39	7.37	7.22	7.59
13	Met	0.73	0.40	0.57	0.29
14	Cys	0.71	2.24	0.80	0.71
15	Ile	5.78	5.80	6.24	6.04
16	Leu	8.18	8.34	8.63	8.69
17	Phe	4.96	5.05	5.07	4.86
18	Lys	6.64	6.59	6.99	7.27

Glycine was the major amino acid found in the all the fruit parts **(Table8, Fig.4a-4d).** Kernel recorded a good amount of Glutamin of 9.77% followed by 9.74% of Glycine, 8.18% of Leucine and Aspargine was noticed. Glycine was higher in aril (11.29%) while methionine was least (0.57%). In testa, Glycine (10.5%) > Proline (8.76%) > Leucine (8.34%) Valine (7.37%) was found in majority. Rind registered higher Glycine of 12.27% and a least Methionine of 0.29%.

Higher Aspargine (8.18%) was found in kernel followed by testa (7.29%). Higher glutamine of 9.77% was registered in kernel while aril recorded least glutamine (6.04%).Testa constituted 6.16% of serine. Glysine was comparatively higher in case of rind (12.27%) followed by aril (11.29%), testa (10.50%) and kernel (9.74%). Histidine, arginine, threonine, tyrosine, methionine, cystine were found in comparatively lesser concentration in all the parts of *M.fatua* fruit. Almost simillar amount of alanine was recorded in kernel (7.18%), testa(7.11%) and rind(7.72%), while aril recorded a least alanine content of 6.53%. Rind exhibited a good percentage of proline of 9.92%. Isoleucine about 5.78-6.24% while leucine ranged between 8.18–8.69%. Phenyl alanine was found in the order of aril (5.07%) > testa(5.05%) > kernel (4.96%)> rind(4.86%). Rind recorded comparatively higher lysine of 7.27% while testa registered least lysine content of 6.59%.

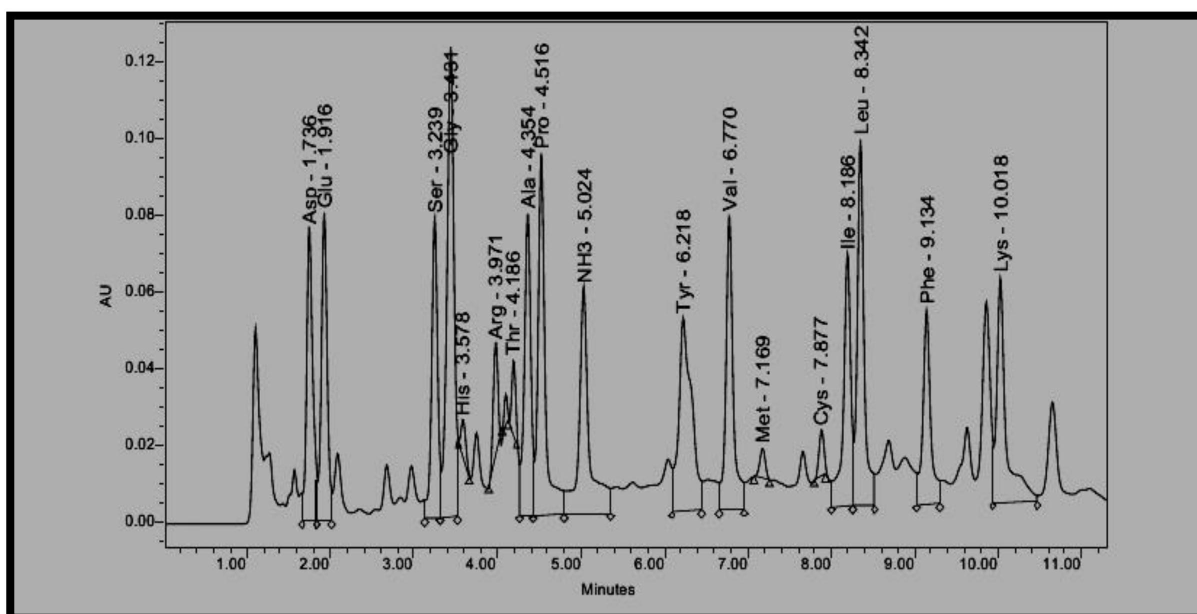


Fig.4a. Amino acid chromatogram of Aril

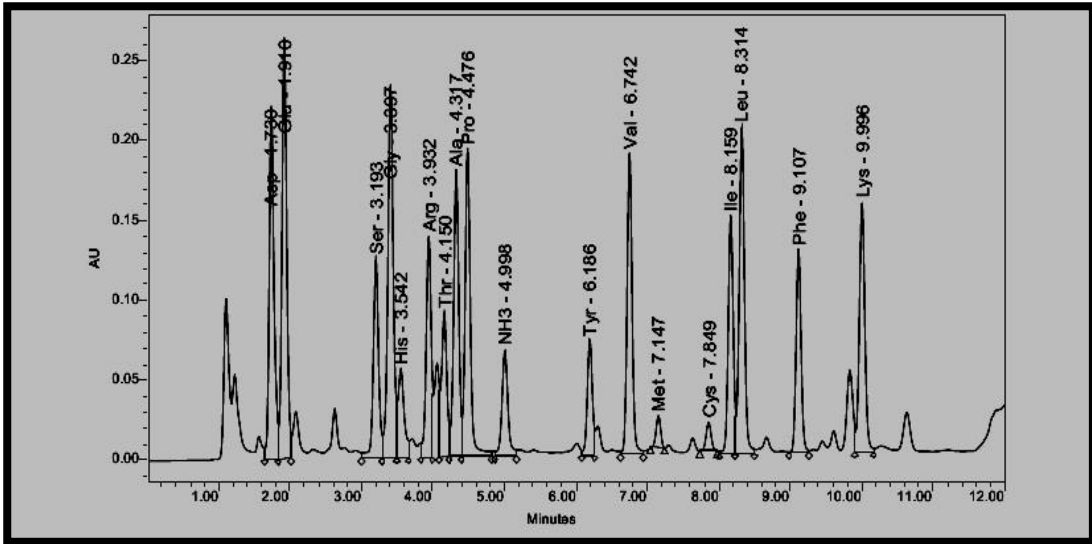


Fig. 4b. Amino acid chromatogram of Kernel

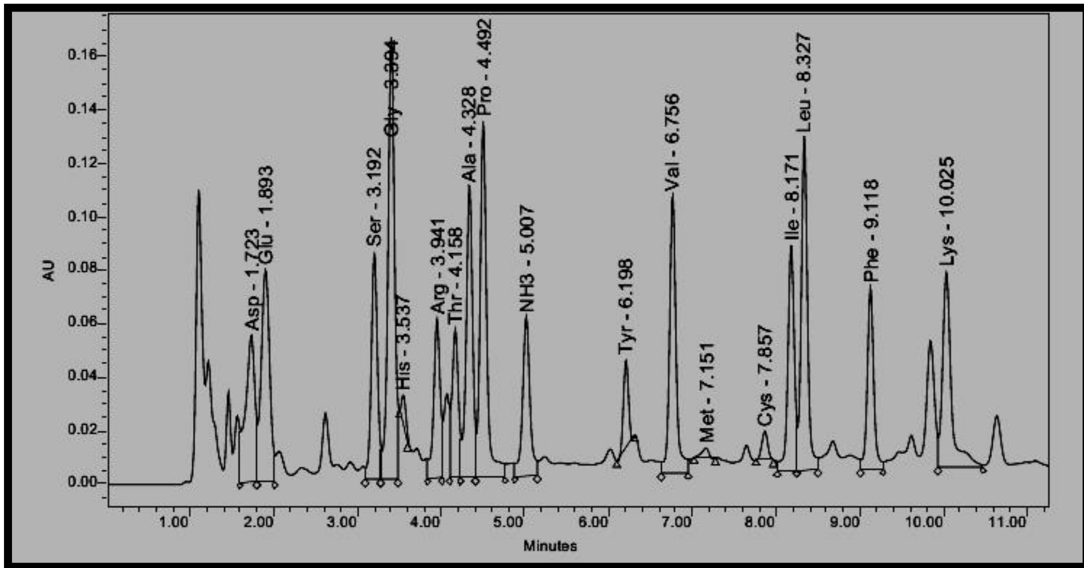


Fig. 4c. Amino acid chromatogram of rind of *M. fatua*

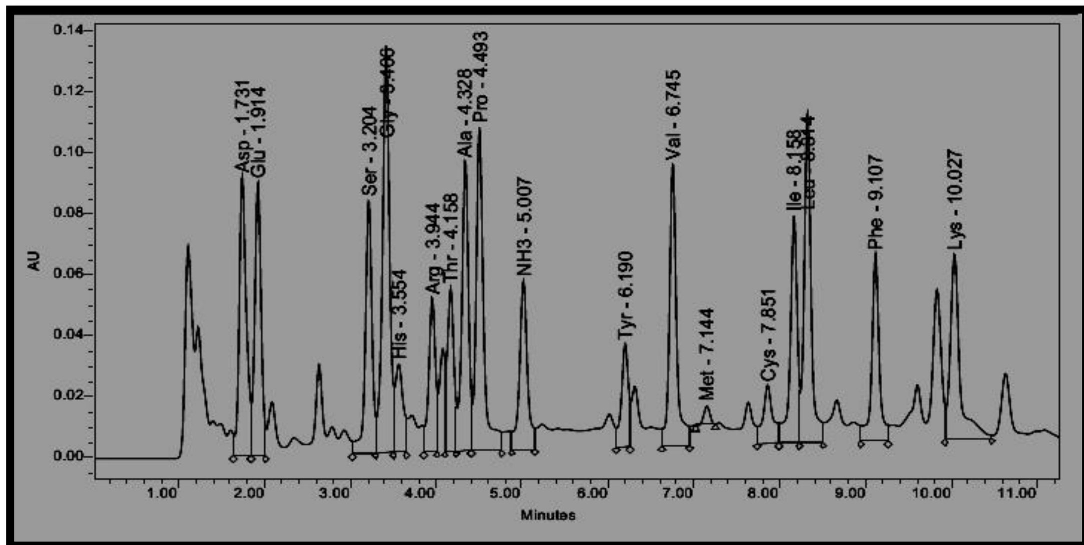


Fig. 4d. Amino acid chromatogram of testa

Table 9. Fatty acid profile of *M. fatua*

Aril	RT	Concentration (%)	Kernel	RT	Concentration (%)
Unidentified	19.595	1.51	Unidentified	19.595	1.56
Unidentified	21.261	4.90	Unidentified	21.261	5.06
Unidentified	21.481	0.74	Colnelenic acid	24.597	57.48
Colnelenic acid	24.597	55.55	Lambertic acid	25.508	1.59
Unidentified	25.508	1.57	Grindelic acid	26.233	31.21
Grindelic acid	26.233	32.90	Unidentified	29.324	1.01
Unidentified	29.324	0.98	Unidentified	32.130	2.09
Unidentified	32.130	1.86	-	-	-

The fatty acid profile of aril and kernel of M. fatua was given in **Table 9.** and **Fig. 5a**.and **5b.** In aril sample, Colnelenic acid (55.55%) and Grindelic acid (32.9%) were the major fatty acids while kernel contained major fatty acids such as Colnelenic acid (57.48%), Lambertic acid (1.59%) and Grindelic acid(31.21%) as shown bellow.

Fig. 5a.Fatty acid profile of aril of *M. fatua*

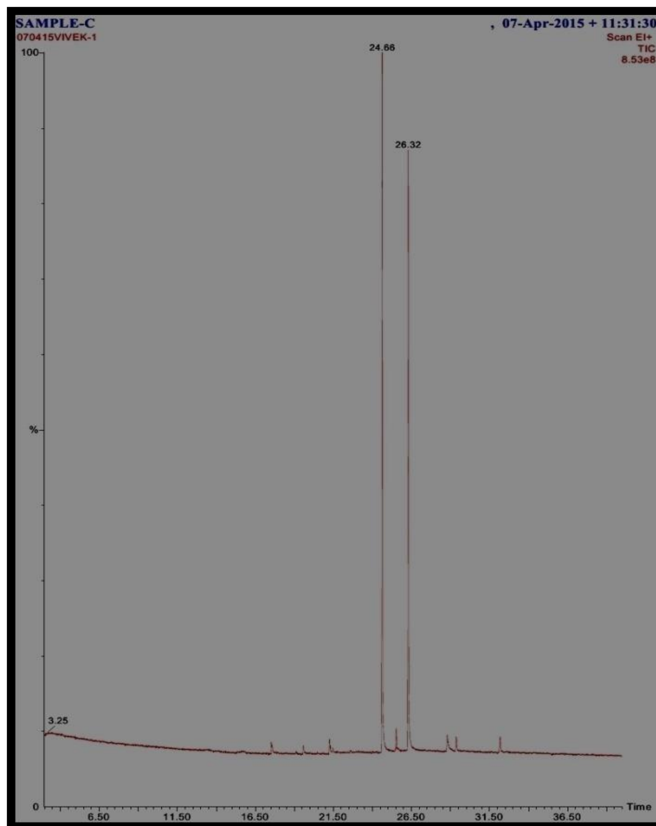


Fig. 5b.Fatty acid profile of kernel of *M. fatua*

Table10. Fatty acid profile of *M. dactyloides*

Kernel	RT	Concentration (%)	Aril	RT	Concentration (%)
Myristic acid	18.11	6.15	Myristic acid	20.110	1.96
Unidentified	18.34	0.35	Oleic acid	21.791	3.66
Palmitic acid	20.12	3.33	Unidentified	21.981	0.34
Oleic acid	21.846	14.31	Unidentified	25.603	25.94
Unidentified	22.001	1.45	Unidentified	25.823	6.87
Malabaricone A	25.593	13.53	Unidentified	25.963	21.52
Unidentified	25.653	0.47	Unidentified	26.168	0.84
Unidentified	25.823	0.76	Unidentified	26.338	0.67
Unidentified	25.928	0.65	Unidentified	26.478	0.83
Unidentified	25.993	0.87	Unidentified	26.543	0.71
Unidentified	26.038	1.87	Unidentified	26.733	2.22
Unidentified	26.183	0.40	Unidentified	27.283	0.98
Unidentified	26.498	0.39	Malabaricone A	29.349	33.45
Unidentified	26.563	0.23	-	-	-
Unidentified	26.758	3.48	-	-	-
Unidentified	27.173	0.53	-	-	-
Paullinic acid	29.404	51.22	-	-	-

The fatty acid profile of aril and kernel of M. dactyloides was depicted in **Table 10.** and **Fig. 6a**.and **6b.** The major fatty acids such as Myristic acid (6.15%), Palmitic acid (3.33%), Oleic acid (14.31%) and Paullinic acid (51.22%) were found in kernel of *M. dactyloides*. Aril sample contained Myristic acid (1.96%), Oleic acid (3.66%) along with 29.34% of resorcinol, Malabaricone A as shown bellow.

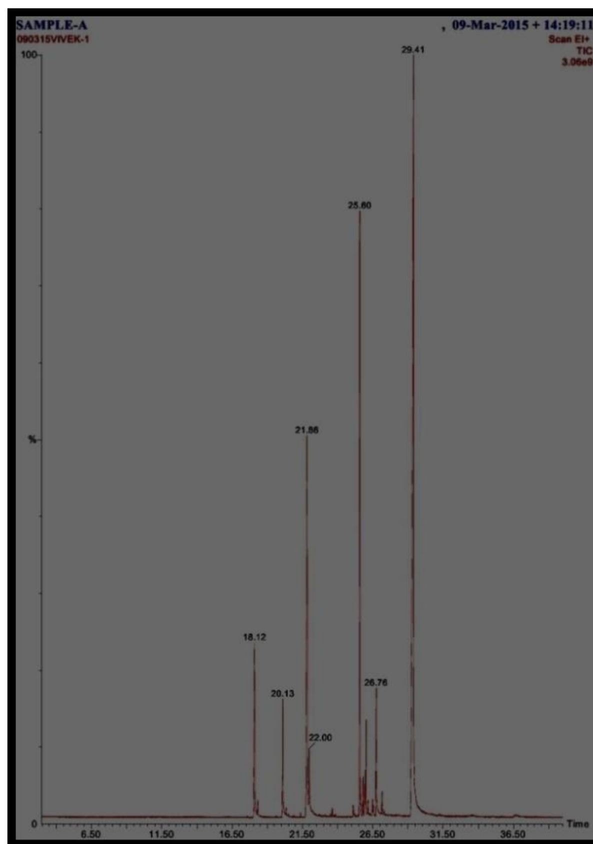


Fig.6a. Fatty acid profile of *M. dactyloides* aril

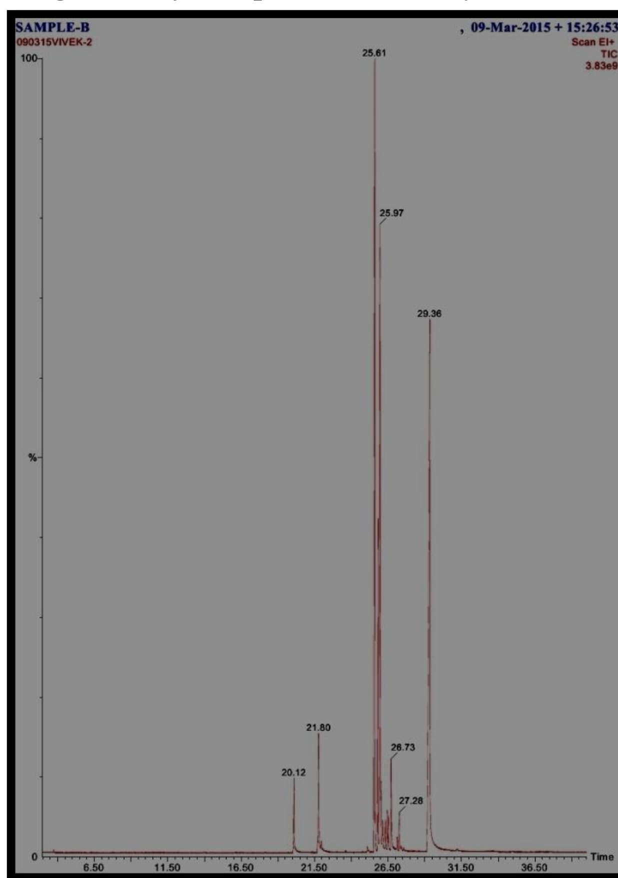


Fig.6b. Fatty acid profile of *M. dactyloides* kernel

Leaf Essential Oil:

There was trace amount essential oil was observed in all the samples such as leaf, kernel, aril and rind in both cases.

Table 11. Phenol, tannin and saponin content of *M. fatua*

Anti-nutritional factors	Kernel	Testa	Aril	Rind	F value
Phenol	137 ± 1^{a}	115.67 ± 0.57^{b}	94 ± 1^{c}	53.67 ± 1.52^{d}	3513.641
Tannin	63 ± 1^{c}	57.67 ± 0.57^{d}	254 ± 1^{a}	124 ± 0^{b}	42890
Saponin	0.54 ± 0.05^{a}	0.7 ± 0.108^{a}	0.171 ± 0.01^{b}	0.31 ± 0.07^{b}	34.373

Table 11. indicates the presence of traces of anti-nutritional factors in different parts of *M. fatua.* Significantly a higher phenolic content was found in *M. fatua* kernel of about 137mg/g. Testa recorded 115.67mg/g of phenol while rind showed comparatively less amount of phenol (53.67mg/g). Aril registered significantly higher tannin of 254mg/g followed by rind (124mg/g), kernel (63mg/g) and testa 57.67). Testa of *M. fatua* exhibited comparatively higher (0.7%) saponin, followed by kernel, 0.54% of saponin content.

Table12. Phenol, tannin and saponin content of *M. dactyloides*

Anti-nutritional factors	Kernel	Testa	Aril	Rind	F value
Phenol	106.33 ± 1.52^{a}	65.67 ± 0.57^{c}	96.67 ± 1.15^{b}	27.33 ± 0.57^{d}	3513.641
Tannin	48 ± 0.5^{d}	52.67 ± 0.30^{c}	117.53 ± 1.35^{b}	124.33^{a}	9091.324
Saponin	0.37 ± 0.04^{a}	0.15 ± 0.03^{b}	0.23 ± 0.02^{b}	0.38 ± 0.02^{a}	38.404

In *M. dactyloides*, kernel registered significantly a higher (106.33mg/g) of phenols while rind exhibited least phenol of 27.33mg/g **(Table 12.)** Tannin was found to be in the order of rind (124.33mg/g) > aril (117.53mg/g) > testa (52.67mg/g) > kernel (48mg/g). In case of *M. dactyloides,* rind recorded comparatively higher saponin while testa showed a least of 0.15% of saponin.

Table 13: Yield of leaf, bark, kernel, aril, testa and fruit rind samples of *M. fatua* and *M. dactyloides* in different solvents.

Plant parts	Extracts	Yield (%)	
		M. fatua	***M. Dactyloides***
Leaf	Water	12%	18.8%
	Methanol	49.2%	9.1%
	Hexane	4.4%	7.2%
	P. ether	4.12%	6.8%
Bark	Water	17%	6.3%
	Methanol	11%	17.3%
	Chloroform	12%	7.9%
	Hexane	2%	8.4%
Kernel	Water	56%	12.9%
	Methanol	19.5%	23.1%
	E. acetate	12%	9.1%
	Chloroform	11%	5.9%
	Hexane	17.36%	16.6%
	P. ether	16%	10%
Aril	Water	12.1%	14%
	Methanol	21%	42%
	E. acetate	75%	30%
	Chloroform	9.9%	11%
	Hexane	13.51%	10.1%
	P. ether	14%	12.4%
Testa	Water	4.8%	5.5%
	Methanol	16%	7.8%
	E. acetate	9%	8.3%
Fruit Rind	Water	5.6%	4.3%
	Methanol	14.2%	5.9%
	E. acetate	10.55%	11%
	Chloroform	8.3%	-

Table 13. Indicates the yield of *Myristica fatua* and *Myristica dactyloides* in different solvents. In *M. fatua*. Ethyl acetate extracts of aril exhibited an highest about 75% followed by water extract of kernel (56%), leaf methanolic extracts (49.2%) and aqueous extract of bark recorded about 17% yield. In case of *M. dactyloides*, comparatively higher yield was noticed in methanolic extract of aril (42%) followed by kernel methonolic extract *(23.1%)* and leaf aqueous extracts (18.8%) and methanolic bark extract exhibited 17.3% of yield. The plant samples extracted in non polar solvents like hexane, chloroform, petroleum ether resulted in comparatively lesser yield.

Table14. Preliminary Phytochemical Screening of different extracts of *M. fatua*

Extract	Alkaloid	Sapo nin	Phen ol	Tan nin	Ster oid	Glyco side	Flavonoid	Resin
MFLAQ	-	+	+	+	-	+	+	-
MFLPE	-	+	+	+	-	+	+	+
MFLH	-	+	+	+	-	+	+	+
MFLM	-	+	+	+	-	+	+	-
MFBM	-	-	+	+	-	+	+	-
MFBCHL	-	-	+	+	-	+	+	-
MFBH	-	-	+	+	-	-	+	-
MFBAQ	-	+	+	+	-	+	+	-
MFAPE	-	-	+	+	-	+	+	-
MFAEA	-	-	+	+	-	+	+	-
MFAM	-	+	+	+	-	-	+	
MFAH	-	-	+	+	-	+	+	+
MFAAQ	-	-	+	+	-	+	+	-
MFACHL	-	+	+	+	-	+	+	+
MFKPE	-	+	+	+	-	+	+	-
MFKEA	-	-	+	+	-	+	+	-
MFKH	-	+	+	+	-	-	+	+
MFKAQ	-	+	+	+	-	+	+	-
MFKM	-	-	+	+	-	+	+	-
MFKCHL	-	-	+	+	-	+	+	-
MFTAQ	-	+	+	+	-	+	+	-
MFTM	-	+	+	+	-	+	+	+
MFTEA	-	+	+	+	-	-	+	-
MFFAQ	-	+	+	+	-	-	+	-
MFFM	-	+	+	+	-	+	+	-
MFFEA	-	-	+	+	-	+	+	-

Table14. Represents the preliminary phytochemical contents of *M. fatua.* Preliminary screening revealed the presence of phenols, tannins and flavonoids while alkaloids and steroids were absent in all the extracts. Saponins were reported in all leaf and testa extracts, aqueous extract of bark, methanolic and chloroform extract of aril, hexane and methanolic extract of kernel and methanolic extract of rind. Glycosides were absent in bark hexane extract, methanolic extract of aril, hexane extract of kernel, ethyl acetate extract of testa and rind aqueous extract. Petroleum ether and hexane extract of leaf, hexane and chloroform extract of aril, hexane extract of kernel and methanolic extract of testa indicated the presence of resins.

Table15. Preliminary Phytochemical Screening of different extracts of *M. dactyloides*

Extract	Alkaloid	Saponin	Phenol	Tannin	Steroid	Glycoside	Flavonoid	Resin
MDL M	-	+	+	+	-	+	+	+
MDL H	-	+	+	+	-	+	+	+
MDL AQ	-	+	+	+	-	+	+	+
MDL PE	-	+	+	+	-	-	+	+
MDB PE	-	-	+	+	+	-	+	+
MDB AQ	-	-	+	+	-	+	+	-
MDB H	-	-	+	+	-	-	+	-
MDB M	-	-	+	+	-	+	+	-
MDK M	-	-	+	+	-	-	+	-
MDK	-	+	+	+	-	+	+	+

CHL								
MDKH	-	+	+	+	-	+	+	-
MDKEA	+	-	+	+	-	+	+	+
MDKAQ	_	-	+	+	-	+	+	-
MDKPE	+	-	+	+	-	-	+	-
MDACHL	_	-	+	+	-	+	+	_
MDAM	-	-	+	+	-	+	+	+
MDAH	-	-	+	+	-	+	+	+
MDAAQ	-	-	+	+	-	+	+	+
MDAPE	-	+	+	+	-	-	+	-
MDTAQ	-	+	+	+	-	+	+	+
MDTM	-	-	+	+	-	-	+	+
MDTEA	-	-	+	+	-	+	+	+
MDFEA	-	-	+	+	-	+	+	+
MDFM	-	-	+	+	-	+	+	+
MDFAQ	-	-	+	+	-	+	+	+

Preliminary screening revealed the presence of phenols, tannins and flavonoids **(Table 15).** Alkaloids were reported in ethyl acetate and petroleum ether extract of kernel while saponin were found in all leaf extracts, hexane and chloroform extracts of kernel, petroleum ether extract of aril and aqueous extract of testa. Steroid was absent except petroleum ether extract of bark. Glycoside was lacking in petroleum ether extract of leaf and bark, hexane extract of bark, methanolic and petroleum ether extract of kernel, petroleum extract of aril and methanolic extract of testa. All the extracts of leaf, testa and rind indicated presence of resins.

Table 16: Phenols and Flavonoid content of different extracts of *M. fatua*.

Plant parts	Extracts	Phenolics	Flavonoids
Leaf	Water	114 ± 0.26^{f}	23.33 ± 0.60^{q}
	Methanol	98.5 ± 0.70^{h}	11 ± 1.10^{s}
	Hexane	114 ± 0.26^{f}	35.33 ± 0.15^{o}
	P. ether	94.5 ± 0.70^{i}	176 ± 0^{f}
Bark	Water	14.67 ± 0.54^{q}	114 ± 0^{j}
	Methanol	93 ± 0.14^{i}	34.67 ± 0.57^{o}
	Chloroform	149.33 ± 0.23^{b}	156.67 ± 0.32^{h}
	Hexane	195 ± 0.26^{a}	177.33 ± 0.15^{f}
Kernel	Water	17 ± 0.14^{q}	30 ± 0^{p}
	Methanol	137 ± 0.66^{c}	11.33 ± 0.15^{s}
	E. acetate	118 ± 0^{e}	53.33 ± 1.1^{m}
	Chloroform	104 ± 0.28^{g}	326 ± 0^{b}
	Hexane	61 ± 0.14^{m}	252 ± 0^{d}
	P. ether	94 ± 0^{i}	274.67 ± 0.30^{c}
Aril	Water	58 ± 0^{n}	124 ± 0.20^{i}
	Methanol	95 ± 0.15^{i}	85.33 ± 0.15^{k}
	E. acetate	64 ± 0^{l}	18 ± 0^{r}
	Chloroform	120 ± 0^{de}	252 ± 0^{d}
	Hexane	43.33 ± 0.33^{o}	8 ± 0^{t}
	P. ether	58 ± 0^{n}	246 ± 0^{e}
Testa	Water	122 ± 0^{d}	46.67 ± 0.15^{n}

	Methanol	118 ± 0^{e}	332 ± 0.60^{a}
	E. acetate	62.67 ± 0.11^{lm}	162 ± 0^{g}
Fruit rind	Water	24.67 ± 0.11^{p}	86 ± 0.2^{k}
	Methanol	55.33 ± 0.21^{n}	20 ± 0^{r}
	E. acetate	68 ± 0^{k}	57 ± 0.1^{l}
	Chloroform	81 ± 0.71^{j}	246.67 ± 0.15^{e}
Means ± SD (n=3) with different alphabets indicate the significant difference at <0.01			

The total phenolics of *M. fatua* ranged between 14.67 -195mg GAE/mg while flavonoids ranged between 8.0-332mg quercetin equivalent/g (**Table16**). Hexane extract of bark showed significantly a highest phenolics of 195mg GAE/g followed by bark chloroform extract (149.33mg GAE/mg) while, water extract recorded least phenolics (14.67mg GAE/mg). A significantly higher amount of flavonoid (332mg quercetin equivalent) was recorded in the methanol extract of testa followed by the chloroform extract of aril (326 mg quercetin equivalent) while hexane extract of aril showed the least (8 mg GAE/mg).

Table 17: Phenols and Flavonoid content of different extracts of *M. dactyloides*.

Plant parts	Extracts	Phenolics	Flavonoids
Leaf	Water	112 ± 1^{bc}	113 ± 1^{fg}
	Methanol	121.67 ± 0.57^{b}	252 ± 0.4^{cd}
	Hexane	$74.67 \pm 2.8^{f-h}$	12 ± 0^{j}
	P. ether	114.6 ± 1.21^{bc}	20 ± 0^{j}
Bark	Water	$71.33 \pm 0.57^{f-h}$	81.33 ± 1.52^{hi}
	Methanol	$76 \pm 1.73^{f-h}$	302 ± 0^{ab}
	Chloroform	172 ± 0^{a}	131 ± 1^{f}
	Hexane	$106.33 \pm 1.52^{b-e}$	135.67 ± 0.37^{f}
Kernel	Water	$39.33 \pm 0.57^{i-k}$	17.67 ± 0.57^{j}
	Methanol	$107 \pm 0.2^{b-d}$	326 ± 1^{a}
	E. acetate	$90 \pm 0^{c-g}$	115.33 ± 0.75^{fg}
	Chloroform	122 ± 0.4^{b}	251.8 ± 0.2^{cd}
	Hexane	$64.67 \pm 0.57^{g-i}$	28.67 ± 0.30^{j}

	P. ether	$84\pm0^{d-h}$	19 ± 1^{j}
Aril	Water	20 ± 1.2^{k}	18.57 ± 0^{j}
	Methanol	$98\pm0^{b-f}$	278 ± 0.91^{bc}
	E. acetate	$94\pm0.6^{b-f}$	292 ± 0.21^{b}
	Chloroform	162 ± 1^{b}	278 ± 0^{bc}
	Hexane	119.67 ± 0.9^{b}	60 ± 1^{i}
	P. ether	$82\pm1^{e-h}$	100 ± 1^{gh}
Testa	Water	35 ± 0.4^{jk}	12 ± 0^{j}
	Methanol	$65\pm1^{g-i}$	252 ± 1^{cd}
	E. acetate	$58\pm0.2^{h-j}$	232 ± 0^{d}
Fruit Rind	Water	16 ± 0^{k}	94 ± 0^{gh}
	Methanol	27 ± 0.4^{k}	77 ± 1^{hi}
	E. acetate	114 ± 0^{bc}	161 ± 1^{e}
Results expressed as Means ± SD (n=3) with different alphabets indicate the significant difference at p<0.01			

The total phenolics of *M. dactyloides* were found in the range of 16-172 mg GAE/mg while flavonoids ranged between 12-326mg quercetin equivalent/g (**Table17**). Chloroform extract of bark showed significantly a highest phenolics of 172mg GAE/g followed by chloroform extracts of aril (162 mg GAE/mg) and kernel (122mg GAE/mg) respectively. Aqueous extract of rind showed least phenolic content of 16mg GAE/mg. A significantly higher amount of flavonoid (326mg quercetin equivalent) was recorded in the methanolic extract of kernel followed by the ethyl acetate extract of aril (292 mg quercetin equivalent) while hexane extract of leaf and aqueous extract of testa showed the least flavonoid content of 8 mg GAE/mg.

Table 18: Antioxidant activity of different extracts of *M. fatua*.

Plant parts	Extracts	Reducing power assay(A700/20 min./100 mg)	DPPH (Concentration @IC_{50} value(mg/ml)
Leaf	Water	5.2±0.2^{u}	0.5933±0.003^{h}
	Methanol	16.85±0.74^{q}	0.6866±0.001^{i}
	Hexane	26.9±0.36^{n}	2.63±0.105^{m}
	P. ether	51.1±0^{i}	0.535±0.005^{g}
Bark	Water	8.9±0.2^{s}	0.125±0.003^{c}
	Methanol	7.8±0.2^{s}	0.049±0.002^{ab}
	Chloroform	6.5±0.5^{t}	0.075±0.003^{a-c}
	Hexane	58.9±0.9^{h}	0.068±0.002^{ab}
Kernel	Water	36.6±0.35^{l}	0.2941±0.004^{e}
	Methanol	81.1±0.1^{c}	0.0355±0.0035^{a}
	E. acetate	90.4±0.37^{b}	0.0324±0.002^{a}
	Chloroform	47.2±0.9^{j}	0.0656±0.004^{ab}
	Hexane	21.2±0.15^{o}	0.10056±0.009^{bc}
	P. ether	78.4±0.36^{d}	0.077±0.006^{a-c}
Aril	Water	65.3±0.5^{g}	0.0496±0.001^{ab}
	Methanol	70.2±0.04^{e}	0.038±0.006^{a}
	E. acetate	40±0^{k}	0.054±0.006^{ab}
	Chloroform	32.3±0.5^{m}	0.0496±0.003^{ab}
	Hexane	68.7±0.35^{f}	0.2068±0.0008^{d}
	P. ether	66.43±0.51^{g}	0.3636±0.0024^{f}
Testa	Water	8.6±0.2^{s}	0.8333±0.012^{j}
	Methanol	68.9±0.05^{f}	0.089±0.005^{a-c}
	E. acetate	65.8±0.7^{g}	0.0315±0.004^{a}
Fruit rind	Water	32±0.1^{m}	0.625±0.008^{h}
	Methanol	14.8±0.66^{r}	1.23±0.045^{k}
	E. acetate	21.2±0.2^{o}	2.02±0.011^{l}
	Chloroform	19.1±0.15^{p}	0.050±0.005^{ab}
Standard Ascorbic acid		116±0.2^{a}	0.070±0.003^{ab}

Results expressed as Means± SD(n=3) with different alphabets indicate the significant

difference at p<0.01

Significantly on par good IC_{50} value were observed in the extracts of bark, kernel, aril and fruit rind **(Table 18)**. Methanolic and ethyl acetate extracts of kernel, methanolic extract of aril and ethyl acetate extract of testa also shown significantly higher on par DPPH activity. However, leaf extracts didn't show any significant DPPH scavenging activity. The IC_{50} values were significantly higher in ethyl acetate extract of testa and kernel, methanolic extract of kernel and aril compared to standard ascorbic acid. A significantly higher reducing power of 90.4mg AAE/g was observed in the ethyl acetate extracts of kernel followed by 81.1, 78.4 and 70.2mgAAE/g was noticed in the kernel, petroleum ether extract of kernel and methanolic extract of aril respectively.

Table 19: Antioxidant activity of different extracts of *M. dactyloides*.

Plant parts	Extracts	Reducing power Assay(A700/20min./100mg)	DPPH (Concentration @IC_{50} value(mg/ml)
Leaf	Water	51.2±0.52^{i}	0.12±0.002^{g}
	Methanol	35.8±0.2^{o}	0.16±0.015^{i}
	Hexane	40.67±0.61^{m}	0.11±0.001^{g}
	P. ether	30.33±0.41^{p}	0.28±0.01^{l}
Bark	Water	43.33±0.41^{k}	0.08±0.003^{f}
	Methanol	36.4±0.40^{o}	0.20±0.0026^{j}
	Chloroform	38.27±0.30^{n}	0.10±0.01^{g}
	Hexane	47.73±0.46^{j}	0.07±0.011^{ef}
Kernel	Water	41.67±0.57^{lm}	0.57±0.003^{n}
	Methanol	73.33±1.15^{c}	0.02±0.007^{a}
	E. acetate	57±1^{h}	0.06±0.005^{cd}
	Chloroform	65.33±0.57^{f}	0.04±0.003^{ab}
	Hexane	42.33±0.57^{kl}	0.65±0.006^{o}
	P. ether	48.33±0.41^{j}	0.28±0.003^{l}
Aril	Water	38.47±0.30^{n}	0.81±0.001^{p}
	Methanol	60.27±0.46^{g}	0.19±0.001^{j}
	E. acetate	80.33±0.57^{a}	0.039±0.003^{ab}
	Chloroform	78.6±0.52^{b}	0.057±0.003^{cd}
	Hexane	67.4±0.4^{e}	0.14±0.006^{h}

	P. ether	65.2 ± 0.2^{f}	0.13 ± 0.006^{h}
Testa	Water	50.93 ± 0.23^{i}	0.57 ± 0.005^{n}
	Methanol	69.8 ± 0.1^{d}	0.060 ± 0.01^{cd}
	E. acetate	70.67 ± 0.57^{d}	0.065 ± 0.005^{de}
Fruit Rind	Water	27.67 ± 0.57^{q}	0.32 ± 0.005^{m}
	Methanol	69.93 ± 0.11^{d}	0.23 ± 0.005^{k}
	E. acetate	50.23 ± 0.05^{i}	0.65 ± 0.01^{o}
Results with different alphabets indicate the significant difference at $p<0.01$			

Significantly a minimum IC_{50} value (0.02mg/ml) was observed in the methanolic extracts of kernel **(Table 19)**. Ethyl acetate extract of aril showed a good IC_{50} value of 0.039mg/ml followed by chloroform extract of kernel (0.04mg/ml) and aril (0.057mg/ml), ethyl acetate extract of kernel (0.06mg/ml) and methanolic extract of testa (0.06mg/ml) was noticed. Comparatively less DPPH free radical scavenging activity was observed in rind extracts (IC_{50} @ 0.23-0.65mg/ml) followed by leaf extracts with IC_{50} value ranging between 0.11-0.16mg/ml. Ethyl acetate extracts of aril exhibited significantly a higher reducing power of 80.33mgAAE/g followed by chloroform extract of aril (78.6 mgAAE/g) and methanolic extract of kernel (73.33mgAAE/g) was noticed.

Table 20: Antibacterial activity of different extracts of *M. fatua*.

Plant parts	Extracts	Zone of inhibition(mm)				
		Gram-positive bacteria		Gram-negative bacteria		
		BS	SA	PV	PA	EC
Leaf	Water	8 ± 0^{g}	$8.3\pm0.1^{d-g}$	10.36 ± 0.11^{e}	10.3 ± 0.1^{c}	7.3 ± 0^{h}
	Methanol	-	-	-	-	-
	Hexane	6.26 ± 0.05^{j}	$8.2\pm0.1^{d-g}$	8.36 ± 0.05^{m}	8.3 ± 0^{h}	8.36 ± 0.05^{g}
	P. ether	6.3 ± 0.05^{j}	$7.3\pm0.1^{f-h}$	8.67 ± 0.11^{kl}	8 ± 0.00^{c}	7.0 ± 0.00^{i}
Bark	Water	9.0 ± 0.00^{f}	$7.3\pm0.1^{f-h}$	9.0 ± 0.0^{j}	5.93 ± 0.3^{l}	11.0 ± 0.0^{b}
	Methanol	-	-	-	-	-
	Chloroform	7.0 ± 00^{i}	$8.6\pm0.00^{d-g}$	7.5 ± 0.1^{n}	9.0 ± 0.0^{e}	9.5 ± 0.3^{e}
	Hexane	6.0 ± 0.0^{k}	$9.0\pm0.0^{b-f}$	10.00 ± 0.0^{f}	9.0 ± 0.0^{e}	11.0 ± 0.0^{b}
Kernel	Water	-	-	7.5 ± 0.8^{n}	-	-

	Methanol	14.7±0.1^{b}	9±0^{b-f}	9.7±0.2^{g}	9±0^{e}	10±0^{d}
	E. acetate	8.67±0.4^{f}	10.6±0.1^{b}	9.3±0.1^{i}	9.0±0.3^{e}	6.67±0.2^{i}
	Chloroform	12±0^{c}	10.5±0.2^{bc}	11.7±0.3^{c}	9.7±0.1^{d}	10±0^{d}
	Hexane	11.67±0^{d}	9.5±0.1^{b-d}	8.4±0.2^{m}	10.7±0.1^{b}	9.3±0.3^{e}
	P. ether	11±0^{e}	9±0^{b-f}	9.5±0.3^{h}	8.5±0^{g}	10.5±0.1^{c}
Aril	Water	9±0^{f}	8.67±0.2^{c-g}	12±0^{b}	6.3±0.3^{k}	10±0^{d}
	Methanol	7±0^{i}	7.5±0.1^{e-h}	8.7±0.1^{k}	7.3±0.3^{j}	7±0^{i}
	E. acetate	7±0^{i}	7.5±0.3^{e-h}	8.5±0.2^{lm}	8.6±0.67^{f}	7±0^{i}
	Chloroform	8±0^{g}	8.5±0.5^{d-g}	9.3±0.1^{i}	8.6±0.67^{f}	9±0^{f}
	Hexane	-	-	-	-	-
	P. ether	7.67±0.7^{h}	9.3±0.1^{b-e}	11±0^{d}	8.6±0.6^{f}	9.0±0^{f}
Testa	Water	-	-	-	-	-
	Methanol	-	6±0^{ij}	6±0^{o}	-	-
	E. acetate	6±0^{k}	8±0.7^{d-g}	6±0^{o}	-	6±0^{j}
Fruit Rind	Water	6±0^{k}	6±0^{ij}	-	6±0^{l}	6±0^{j}
	Methanol	-	-	-	-	-
	E. acetate	7±0^{i}	7±0^{g-i}	6±0^{o}	-	6±0^{j}
	Chloroform	6.1±0.1^{k}	7.1±0.1^{f-h}	-	-	6±0^{j}
Standard Tetracycline		24.1±0.2^{a}	22±0.00^{a}	23±0.1^{a}	22.4±0.1^{a}	21.8±0.1^{a}

The diameter of Zone of Inhibition (mm) including disc diameter of 5mm.

Results with different alphabets indicate the significant difference at $p<0.01$

BS-*Bacillus subtilis*, SA-*Staphylococcus aureus*, PV- *Proteus vulgaris*, PA-*Pseudomonas aeruginosa,* EC- *Escherichia coli*,-No activity

Methanolic extract of kernel showed a good antibacterial activity against *Bacillus subtilis* which was significantly higher compared to other extracts. A significantly higher antibacterial activity was noticed in case of ethyl acetate extract of kernel. The water extract of aril showed a significantly higher inhibition zone of 12mm which is almost the half of the inhibition zone shown by standard tetracycline against *Proteus vulgaris*.The chloroform extract of kernel, petroleum ether extract of aril and water extract of leaf showed significantly a good zone of inhibition.

The hexane extract of the kernel followed by water extract of leaf recorded a good antibacterial activity against *Pseudomonas aeruginosa* while water and hexane

extract of bark exhibited on par higher antibacterial activity against *E-coli*. However, the antibacterial activity of the crude extract was more than 50 times low compared to standard drug Tetracycline.

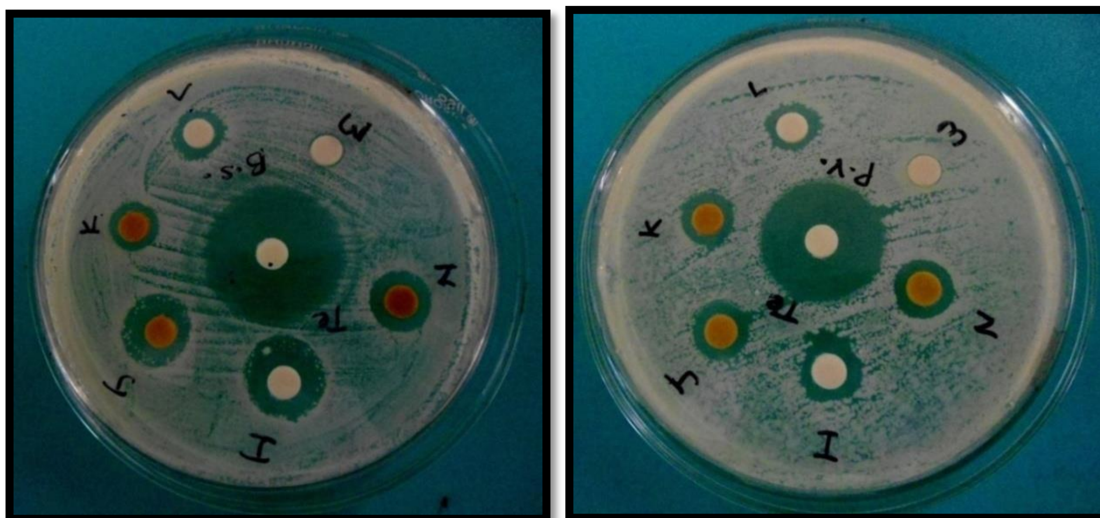


Fig.7a. **Fig.7b.**

Fig.7a. & 7b. Antibacterial activity of *M. fatua* aril extracts towards *B. subtilis* and *P. vulgaris*

Where, I- *M.fatua* aril pet. Ether extract, J- *M. fatua* ethyl acetate extract, K- *M. fatua* aril methanolic extract, L- *M. fatua* aril hexane extract, M- *M. fatua* aril aqueous extract, N- *M. fatua* aril chloroform extract, Te- standard Tetracycline.

Table 21: Minimum Inhibitory Concentration

Part	**Extract**	**Bacteria**	**MIC(mg)**
Aril	P. ether	*Bacillus subtilis*	1.25
Aril	Methanol	*Bacillus subtilis*	10
Aril	E. acetate	*Bacillus subtilis*	2.5
Aril	Chloroform	*Staphylococcus aureus*	2.5
Kernel	Methanol	*Staphylococcus aureus*	1.25
Standard Tetracycline		*Bacillus subtilis*	0.0025
Standard Tetracycline		*Staphylococcus aureus*	0.0025

Petroleum ether extract of aril inhibited the visible growth of *Bacillus subtilis* at a MIC of 1.25mg (**Table 21).** Similarly, the methanol extract of kernel showed an inhibition of *Staphylococcus aureus* at the concentration of 1.25mg (**Fig.8**).

Fig.8. Minimum inhibitory concentration of of *M. fatua* kernel methanolic extracts towards *Staphylococcus aureus.*

Table 22. Minimum Bactericidal Concentration

Part	Extract	Bacteria	MBC(mg)
Aril	Chloroform	*Staphylococcus aureus*	5
Kernel	Methanol	*Staphylococcus aureus*	10
Standard Tetracycline		*Staphylococcus aureus*	0.0025

The MBC assay revealed the minimum bactericidal concentration of 5mg and10 mg of the chloroform extract of aril and methanolic extract of kernel respectively to show bactericidal activity while standard Tetracycline showed minimum bactericidal concentration of 0.0025mg against *Staphylococcus aureus.* (**Table 22**).

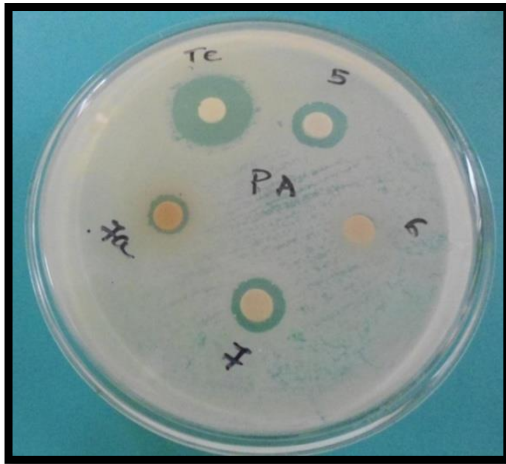
Table 23: Antibacterial activity of different extracts of *M. dactyloides*.

Plant parts	Extracts	Zone of Inhibition(mm)				
		Gram positive bacteria		Gram Negative bacteria		
		BS	SA	PV	PA	EC
Leaf	Water	-	-	-	-	-
	Methanol	-	7±0^{f}	-	6.3±0.3^{h}	6±0^{i}
	Hexane	7±0.1^{h}	6.7±0.5^{g}	6±0^{h}	8.3±0.5^{f}	6.7±0.3^{h}
	P. ether	6±0^{i}	6±0.2^{g}	6.3±0.1^{h}	-	6.33±0.1^{i}
Bark	Water	-	-	-	-	-
	Methanol	8.3±0.3^{g}	7±0^{f}	10.3±0.1^{d}	7.3±0.6^{g}	8±0^{f}
	Chloroform	13.3±0.1^{b}	12.6±0^{b}	12±0^{b}	12±0^{b}	11.7±0.1^{c}
	Hexane	12.3±0^{c}	11.3±0^{c}	11±0^{c}	12.3±0.1^{b}	12.3±0.2^{b}
Kernel	Water	-	-	6±0^{h}	-	-
	Methanol	11.3±0.1^{d}	10.3±0^{d}	11.3±0.1^{c}	8.67±0.1^{f}	9.3±0.3^{e}
	E. acetate	9.6±0^{f}	9.6±0.5^{d}	10±0^{d}	11±0^{c}	9.67±0.2^{de}
	Chloroform	9.3±0.2^{f}	9.6±0.2^{d}	10.3±0.1^{d}	8.7±0.2^{f}	10.3±0.1^{d}
	Hexane	10±0.1^{e}	8.3±0.1^{e}	6±0^{h}	11.3±0.1^{c}	7.67±0.3^{g}
	P. ether	8±0^{g}	-	-	7.3±0.2^{g}	7±0^{g}
Aril	Water	11±0.1^{d}	-	-	-	-
	Methanol	12.3±0^{c}	8.67±0.1^{e}	8±0^{f}	9±0.31^{e}	11±0^{c}
	E. acetate	12.6±0.2^{c}	9.67±0.2^{d}	8±0^{f}	8.3±0.4^{f}	10±0^{d}
	Chloroform	13.3±0^{b}	12±0^{bc}	9±0^{e}	9.3±0.23^{e}	9.6±0.1^{de}
	Hexane	12.3±0^{c}	8.3±0.5^{e}	9±0^{e}	8.67±0.1^{f}	7±0^{g}
	P. ether	9±0^{f}	7.3±0.1^{f}	7±0^{g}	9±0^{e}	7±0^{g}
Testa	Water	-	-	-	-	-
	Methanol	8±0.7^{g}	8.7±0.2^{e}	8±0.5^{f}	-	8±0^{f}
	E. acetate	8±0.1^{g}	7.3±0.3^{f}	8.5±0.3^{f}	-	9±0^{e}
Fruit rind	Water	-	-	-	-	-
	Methanol	10.7±0^{e}	11±0^{c}	11±0^{c}	11.33±0.1^{c}	-
	E. acetate	12.3±0^{c}	12±0^{bc}	11±0^{c}	10±0^{d}	-
Standard Tetracycline		24.1±0.2^{a}	22±0.00^{a}	23±0.1^{a}	22.4±0.1^{a}	21.8±0.1^{a}

Diameter of Zone of Inhibition (mm) including disc diameter of 5mm.
Results with different alphabets indicate the significant difference at $p<0.01$
BS-*Bacillus subtilis*, SA-*Staphylococcus aureus*, PV- *Proteus vulgaris*, PA-*Pseudomonas*

aeruginosa, EC- *Escherichia coli*,-No activity

Chloroform extract of bark showed comparatively a good antibacterial activity against *Bacillus subtilis* which was comparatively higher than other extracts **(Table 23).** A comparatively higher antibacterial activity was noticed in case of chloroform extract of bark against *Staphylococcus aureus* with an inhibitory zone of 12.6mm. *Proteus vulgaris* was inhibited by chloroform extract of bark with an inhibitory zone of 12mm while aqueous extract of leaf, bark, testa and rind completely lacked the antibacterial activity. However, antibacterial activities of crude extracts were lower than the standard Tetracycline.



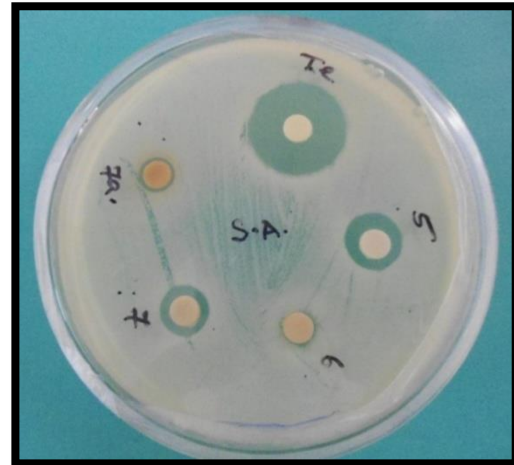
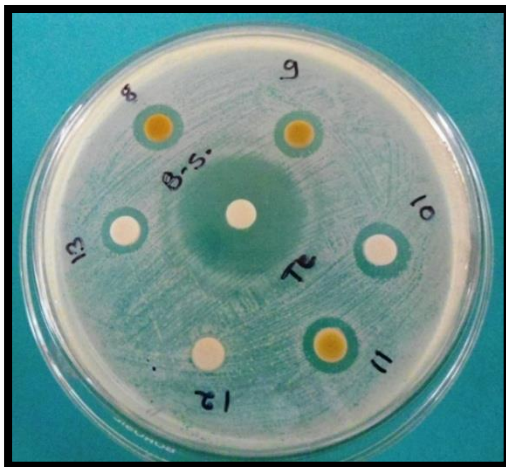


Fig.9a. **Fig.9b.**

Fig.9a. & 9b. Antibacterial activity of *M. dactyloides* bark extracts towards *P. vulgaris* and *Staphylococcus aureus* Where, 5-pet.ether extract, 6- aqueous extract, 7- hexane extract, 8- methanolic extract, Te- standard Tetracycline.



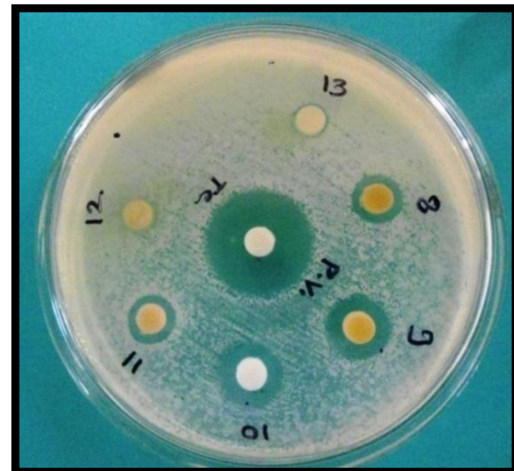


Fig. 10a. **Fig. 10b.**

Fig.10a. & 10b. Antibacterial activity of *M. dactyloides* kernel extracts towards *B. subtilis* and *P. vulgaris*.

Where, 8-methanolic extract, 9- chloroform extract, 10- hexane extract, 11- ethyl acetate extract, 12- aqueous extract, 13- pet.ether extract, Te- standard Tetracycline.

Table 24: Minimum Inhibitory Concentration

Part	Extract	Bacteria	MIC(mg)
Bark	P. ether	*B. subtilis*	10
Bark	Hexane	*S. aureus*	2.5
Bark	Hexane	*P. vulgaris*	1.25
Bark	Hexane	*P. aurigenosa*	1.25
Bark	P. ether	*E.Coli*	10
Bark	Hexane	*E-Coli*	5
Aril	Ethyl acetate	*B. subtilis*	1.25
Aril	Chloroform	*B. subtilis*	6.25
Aril	Methanol	*B. subtilis*	5
Aril	Hexane	*B. subtilis*	1.25
Standard Tetracycline		*P. vulgaris*	0.0025
Standard Tetracycline		*P. aurigenosa*	0.0025
Standard Tetracycline		*E-Coli*	0.0025
Standard Tetracycline		*Bacillus subtilis*	0.0025
Standard Tetracycline		*S. aureus*	0.0025

Petroleum ether extract of bark inhibited the visible growth of *Bacillus subtilis* at a MIC of 10mg **(Table 24).** Similarly, the hexane extract of bark showed inhibition on *Staphylococcus aureus, Proteus vulgaris* and *Pseudomonas aurigenosa* at the concentration of 2.5, 1.25 and 1.25mg respectively. The visible growth of *E-Coli* was inhibited by petroleum ether and hexane extract of bark with MIC of 10 and 5mg respectively. *Bacillus subtilis* was inhibited by the ethyl acetate, chloroform, methanol and hexane extract of aril with MIC of 1.25, 6.25, 5 and 1.25mg respectively.

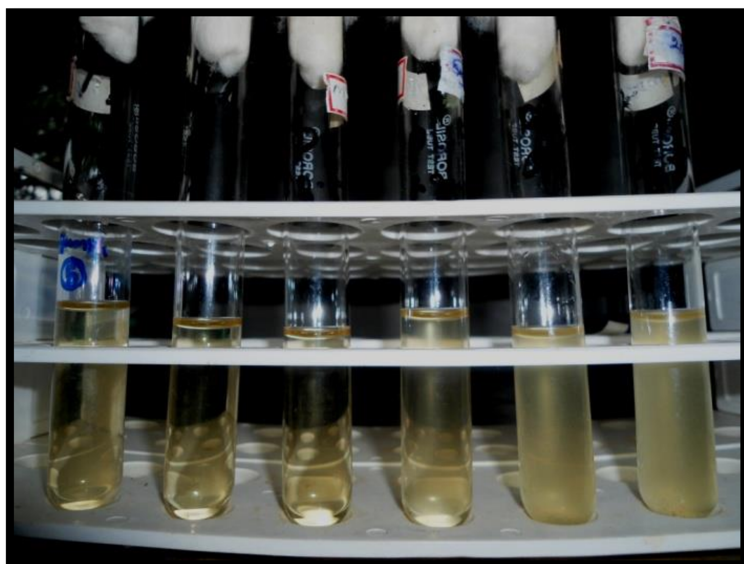


Fig.11. Minimum inhibitory concentration of *M. fatua* bark pet. ether extracts towards *Proteus vulgaris.*

Table 25. Minimum Bactericidal Concentration

Part	Extract	Bacteria	MBC(mg)
Bark	Hexane	*Proteus vulgaris*	5
Aril	Chloroform	*Staphylococcus aureus*	5
Aril	Hexane	*Bacillus subtillus*	10
Standard Tetracycline		*Staphylococcus aureus*	0.0025

The MBC assay reveals the minimum bactericidal concentration of bark and aril extracts of *M. dactyloides* (**Table 25**). From the table, it was clear that hexane extract of bark showed minimum bactericidal activity at the concentration of 5mg against *Proteus vulgaris.* Chloroform and hexane extract of aril exhibited minimum bactericidal activity at the concentration of 5 and 10mg towards *Staphylococcus aureus, Bacillus subtillus* respectively.

Table 26. ***In- vitro*** **α-amylase activity of different extracts of** ***M. fatua*** **at the concentrations of 20 and 10µg.**

Plant parts	Extracts	α-amylase Inhibitory activity (%)at 20µg	α-amylase Inhibitory activity (%)at 10µg
Leaf	Water	-	-
	Methanol	87.83±0.5^{e}	56.58±0.4^{o}
	Hexane	85.1±0.7^{f}	75.7±0.3^{i}
	P. ether	-	-
Bark	Water	90.63±0.3^{c}	65.43±0.5^{m}
	Methanol	91.24±0.2^{b}	59.46±0.3^{n}
	Chloroform	-	-
	Hexane	-	-
Kernel	Water	91.56±0.9^{b}	82.11±0.1^{g}
	Methanol	75.17±0.3^{i}	86.76±0.6^{e}
	E. acetate	91.8±0.6^{b}	88.53±0.3^{d}
	Chloroform	81.35±0.4^{g}	82.5±0.5^{g}
	Hexane	91.06±0.1^{b}	84.33±0.3^{f}
	P. ether	84.31±0.4^{e}	93.3±0.3^{b}
Aril	Water	50.33±0.2^{o}	79.36±0.4^{h}
	Methanol	52.83±0.1^{n}	74.36±0.3^{j}
	E. acetate	74.5±0.1^{j}	56.3±0.3^{o}
	Chloroform	68.01±0.2^{m}	86.83±0.3^{e}
	Hexane	68.23±0.2^{m}	85.56±0.7^{e}
	P. ether	68.46±0.7^{m}	68±0.2^{l}
Testa	Water	85.46±0.5^{e}	92.2±0.2^{c}
	Methanol	86.6±0.7^{d}	95.36±0.3^{a}
	E. acetate	71.07±0.1^{l}	69±0.4^{k}
Fruit rind	Water	83.16±0.4^{f}	84.33±0.1^{e}
	Methanol	77.86±0.2^{h}	88.1±0.2^{d}
	E. acetate	72.33±0.3^{k}	85.4±0.2^{e}
	Chloroform	21.86±0.4^{p}	-
Standard Acarbose		92.13±0.2^{a}	84.38±0.1^{e}

Results with different alphabets indicate the significant difference at $p<0.01$

At 20µg, on par α-amylase inhibitory activity was exhibited by ethyl acetate extract (91.8%) and aqueous extract of kernel(91.56%) followed by methanolic extract of bark (91.24%) and hexane extract of kernel(91.06%) which is comparable with the standard Acarbose (92.13%)**(Table 26).** Significantly a higher amount (95.36%) of α-amylase inhibitory activity was seen in case of methanolic extract of testa at the concentration of 10µg, followed by petroleum ether extract of kernel (93.3%), aqueous extract of testa (92.2%) and on par inhibition of 88.53 & 88.1% was registered in ethyl acetate extract of kernel and methanolic extract of rind.

Table 27: *In -vitro* α-glucosidase activity of different extracts of *M. fatua* at the concentrations of 20 and 10µg.

Plant parts	Extracts	α-glucosidase Inhibitory activity (%)at 20µg	α-glucosidase Inhibitory activity (%)at 10µg
Leaf	Water	81.15±0.8[d]	40.95±0.5[k]
	Methanol	-	-
	Hexane	64.67±0.3[f]	72.08±0.6[b]
	P. ether	51.47±0.6[j]	70.46±0.7[d]
Bark	Water	-	71.28±0.7[c]
	Methanol	-	-
	Chloroform	53.89±0.2[i]	66.61±0.8[e]
	Hexane	50.21±0.2[k]	71.28±0.7[c]
Kernel	Water	61.18±0.1[g]	50.77±0.1[j]
	Methanol	66.11±0.6[e]	72±0.2[c]
	E. acetate	96.73±0.5[a]	53.26±0.3[i]
	Chloroform	-	-
	Hexane	44.75±0.4[m]	44.79±0.5[k]
	P. ether	44.36±0.7[m]	40.83±0.1[l]
Aril	Water	-	-
	Methanol	-	-
	E. acetate	83.64±0.9[c]	65.28±0.8[f]
	Chloroform	-	-
	Hexane	40.7±0.5[n]	69.12±0.9[d]

	P. ether	46.2±0.1^{l}	62.94±0.6^{g}
Testa	Water	19.54±0.8^{o}	26.46±0.1^{n}
	Methanol	-	-
	E. acetate	-	-
Fruit rind	Water	55.44±0.2^{h}	32.5±0.1^{m}
	Methanol	-	-
	E. acetate	-	-
	Chloroform	51.73±0.3^{j}	58.16±0.6^{h}
Standard Acarbose		90.33±0.5^{b}	90.41±0.3^{a}
Results with different alphabets indicate the significant difference at $p<0.01$			

Significantly a higher α-glucosidase inhibitory activity was registered in ethyl acetate extract of kernel (96.73%), followed by standard Acarbose (90.33%) at the concentration of 20µg as shown in **Table 27**. But in case of 10µg, standard Acarbose exhibited significantly higher inhibitory action against α-glucosidase enzyme followed by hexane extract of leaf recorded 72.08 % of inhibition while methanolic extract of leaf, water and methanolic extract of bark, chloroform extract of kernel, aqueous, methanolic and chloroform extract of aril, methanolic and ethyl acetate extract of testa and rind completely lacked α-glucosidase inhibitory activity at both the concentration.

Table 28: *In- vitro* α-amylase activity of different extracts of *M. dactyloides* at the concentrations of 20 and 10µg.

Parts	Extract	α- Amylase activity @ 20µg (%)	α- Amylase activity @ 10µg (%)
Leaf	Methanol	57.5±0.45^{l}	37.5±0.3^{j}
	Hexane	-	-
	Water	-	-
	Petroleum ether	-	-
Bark	Chloroform	-	-
	Water	93.77±0.55^{a}	44.8±0.41^{i}
	Hexane	-	-
	Methanol	42.7±0.4^{n}	-
Kernel	Methanol	79.03±0.4^{d}	71.41±0.24^{ef}
	Chloroform	53.1±0.5^{m}	76.23±0.32^{d}

	Hexane	64.23±0.83^{i}	72.4±0.4^{e}
	Ethyl acetate	77.63±0.45^{e}	81.33±0.41^{b}
	Water	65.64±0.78^{h}	75.3±0.26^{d}
	Petroleum ether	91.70±0.39^{b}	93.13±0.15^{a}
Aril	Ethyl acetate	-	-
	Chloroform	-	-
	Methanol	-	-
	Hexane	62.16±0.6^{j}	73.36±0.35^{e}
	Water	59.36±0.51^{k}	71.03±0.15^{f}
	Petroleum ether	83.7±0.45^{c}	98.4±0.26^{a}
Testa	Water	68.8±0.78^{g}	-
	Methanol	-	-
	Ethyl acetate	22.43±0.32^{o}	-
Fruit rind	Ethyl acetate	61.066±0.25^{k}	77.26±0.2^{c}
	Methanol	71.43±0.32^{f}	69.1±0.26^{g}
	Water	92.63±0.47^{b}	59.83±0.55^{h}
Acarbose		92.13±0.21	84.38±0.13
Results with different alphabets indicate the significant difference at $p<0.01$			

Significantly a higher amount (93.7% &) of α-amylase inhibitory activity was seen in case of water extract of bark at the concentration of 20µg which was on par with that of the standard acarbose **(Table 28).** About 91.5% inhibition was shown by petroleum ether extract of kernel while ethyl acetate extract of testa recorded least. Among fruit rind extracts, water extract exhibited a good percentage of inhibition of 92.6% against α-amylase enzyme.

About 83.7% of inhibition was noticed in case of aril petroleum ether extract. Significantly a good percentage of 98.43% inhibition against α-amylase was seen by aril petroleum ether extract at the concentration of 10µg.

Table 29: *In -vitro* α-glucosidase activity of different extracts of *M. dactyloids* at the concentrations of 20 and 10µg.

Parts	Extract	α- glucosidase activity @ 20µg (%)	α- glucosidase activity@ 10µg (%)
Leaf	Methanol	-	-
	Hexane	46.88±0.78^{d}	49.58±0.5^{a}
	Water	49.49±0.59^{c}	37.91±0.7^{e}
	Petroleum ether	51.63±0.62^{b}	46.96±0.78^{b}
Bark	Chloroform	46.58±0.38^{d}	38.59±0.27^{de}
	Water	68.59±0.67^{a}	35.69±0.45^{f}
	Hexane	-	-
	Methanol	-	-
Kernel	Methanol	-	-
	Chloroform	-	-
	Hexane	46.72±0.96^{d}	39.22±0.2^{d}
	Ethyl acetate	-	-
	Water	17.94±0.47^{h}	-
	Petroleum ether	48.88±0.55^{c}	47.33±0.31^{b}
Aril	Ethyl acetate	-	-
	Chloroform	-	-
	Methanol	-	-
	Hexane	42.68±0.32^{e}	50.11±0.1^{a}
	Water	28.3±0.91^{g}	-
	Petroleum ether	41.66±0.62^{f}	45.03±0.61^{c}
Testa	Water	17.03±0.1^{h}	32.23±0.15^{g}
	Methanol	-	-
	Ethyl acetate	-	-
Fruit rind	Ethyl acetate	-	-
	Methanol	-	-
	Water	-	-
Acarbose		90.33±0.57	90.41±0.37

Results with different alphabets indicate the significant difference at $p<0.01$

Significantly a higher amount of (68.7%) α-glucosidase enzyme inhibitory activity was seen in the case of water extract of bark at 20µg as shown in **Table 29.** Leaf methanolic extract showed 51.6% of inhibition while water extract of testa showed the least inhibition. Methanolic extract of the aril showed higher inhibition about 50.13% while both the fruit rind and testa lack the inhibitory activity at 10µg.

Table 30: *In -vitro* anti inflammatory activity of different extracts of *M. fatua* at the concentrations of 20 and 10µg.

Plant parts	Extracts	Inhibitory activity (%)at 20µg	Inhibitory activity (%)at 10µg
Leaf	Water	88.59 ± 0.45^{b}	88.2 ± 0.6^{b}
	Methanol	78.56 ± 0.83^{d}	78.56 ± 0.51^{d}
	Hexane	14.63 ± 0.47^{r}	14.63 ± 0.32^{q}
	P. ether	41.3 ± 0.60^{m}	41.33 ± 0.57^{l}
Bark	Water	72 ± 0.1^{g}	71 ± 1^{f}
	Methanol	35.2 ± 0.46^{n}	30.5 ± 0.5^{p}
	Chloroform	70 ± 0.81^{g}	66.67 ± 0.28^{h}
	Hexane	45.33 ± 0.57^{l}	45.83 ± 0.2^{k}
Kernel	Water	76.79 ± 0.74^{e}	76.33 ± 0.32^{e}
	Methanol	65.26 ± 0.30^{i}	65.33 ± 0.2^{h}
	E. acetate	54.5 ± 0.86^{k}	52 ± 1^{j}
	Chloroform	54.5 ± 0.50^{k}	54.33 ± 0.57^{j}
	Hexane	76.5 ± 0.50^{f}	90.33 ± 0.57^{a}
	P. ether	44.66 ± 0.83^{l}	45 ± 1^{k}
Aril	Water	69.2 ± 0.3^{h}	66.67 ± 0.57^{h}
	Methanol	82.8 ± 0.9^{c}	81.5 ± 0.62^{c}
	E. acetate	90 ± 1.08^{a}	90 ± 0^{a}
	Chloroform	88.16 ± 0.79^{b}	34.67 ± 0.57^{n}
	Hexane	30.03 ± 0.39^{p}	54.62 ± 0.61^{j}
	P. ether	17 ± 1^{q}	10 ± 0^{r}
Testa	Water	62.83 ± 0.20^{j}	62.5 ± 0.5^{i}
	Methanol	69.5 ± 0.50^{h}	68.33 ± 0.57^{g}

	E. acetate	32±0^{o}	32.16±0.76^{o}
Fruit rind	Water	40.8±0.43^{m}	40.67±0.57^{l}
	Methanol	70.96±0.45^{g}	70.67±0.23^{f}
	E. acetate	44.43±0.51^{l}	44.67±0.5^{k}
	Chloroform	37.26±0.20^{n}	37.5±0.5^{m}
Standard Acarbose		82.4±1^{c}	79.2±0.1^{d}
Results with different alphabets indicate the significant difference at p<0.01			

Significantly a higher anti-inflammatory activity was shown by ethyl acetate extract of aril (90±1.08%) at 20µg followed by aqueous extract of leaf (88.59±0.45%) and chloroform extract of aril (88.16%) which was significantly higher than the anti-inflammatory property exhibited by standard diclofenac(82.4±1%) as shown in **Table 30.** Similarly at 10µg concentration, ethyl acetate extract of aril exhibited significantly higher anti-inflammatory property of about 90% followed by aqueous extract of leaf (88.2%), methanolic extract of aril (81.5%) respectively while standard diclofenac showed about 79.2% inhibition towards inflammation.

Table 31. ***In -vitro*** **anti inflammatory activity of different extracts of** ***M. fatua*** **at the concentrations of 20 and 10µg.**

Sample	**Extracts**	**α- inflammatory activity @ 10µg (%)**
Leaf	Methanol	-
	Hexane	-
	Water	94.93±0.11^{a}
	Petroleum ether	82.46±0.61^{c}
Bark	Chloroform	38±0.1^{p}
	Water	76.1±1^{e}
	Hexane	-
	Methanol	-
Kernel	Methanol	56.8±0.8^{k}
	Chloroform	50±0^{m}

	Hexane	88 ± 1^{b}
	Ethyl acetate	68.3 ± 0.4^{h}
	Water	53.66 ± 0.5^{l}
	Petroleum ether	40.13 ± 0.8^{o}
Aril	Ethyl acetate	71 ± 0^{g}
	Chloroform	44 ± 1^{n}
	Methanol	80 ± 0^{d}
	Hexane	51 ± 0.4^{m}
	Water	63 ± 0^{i}
	Petroleum ether	-
Testa	Water	68.13 ± 0.5^{h}
	Methanol	73 ± 0^{f}
	Ethyl acetate	-
Fruit rind	Ethyl acetate	-
	Methanol	27.83 ± 0.28^{q}
	Water	58.33 ± 0.57^{j}
Sodium diclofenac		79.2 ± 0.1
Results with different alphabets indicate the significant difference at $p<0.01$		

Significantly a higher anti-inflammatory potential was observed in water extract of leaf followed by 88% and 82.4% in case of hexane extract of kernel and petroleum ether extract of leaf respectively (**Table 31).** Also, it is found that methanolic aril extract inhibited the inflammation to the extent of about 80% which is on par with that of the standard sodium diclofenac.

Acute toxicity

There was no mortality found even after 72 hours indicating that the ethyl acetate extract is safe up to a single dose of 2000mg/kg body weight. Therefore, therapeutic doses such as 100, 200 and 400mg/kg body weight were selected for testing the analgesic activity and the dose which had shown higher analgesic activity was chosen for anti-inflammatory, anti-hyperlipidemic studies, and anti-diabetic studies. There was no death till the end of the study.

Table 32. **Analgesic activity of the Ethyl acetate extract of *M. fatua* using hot plate method in mice.**

Group	Time Interval (Min.)				
	0	**30**	**60**	**90**	**120**
	Reaction time (Sec)				
1. Control	1.975 ± 0.09^{a}	2.05 ± 0.05^{d}	2.075 ± 0.12^{d}	2.0 ± 0^{e}	2.05 ± 0.1^{e}
2. Pentazocine (10mg/kg)	2 ± 0.08^{a}	4.5 ± 0.50^{a}	5.61 ± 0.54^{a}	6.05 ± 0.12^{a}	7.05 ± 0.09^{a}
3.MFAEA (100 mg/kg)	1.925 ± 0.05^{a}	3.02 ± 0.05^{c}	3.605 ± 0.17^{c}	3.075 ± 0.09^{d}	2.87 ± 0.098^{d}
4.MFAEA (200 mg/kg)	1.925 ± 0.05^{a}	3.65 ± 0.4^{bc}	4.125 ± 0.18^{bc}	3.8 ± 0.11^{c}	3.76 ± 0.04^{c}
5.MFAEA (400 mg/kg)	1.975 ± 0.05^{a}	3.94 ± 0.02^{ab}	4.625 ± 0.17^{b}	4.33 ± 0.40^{b}	4.05 ± 0.1^{b}
F value	0.964	36.87	85.43	222.21	406.57

Table 32. indicates the analgesic activity of ethyl acetate extracts using hot plate method in Swiss albino mice. Ethyl acetate extract of *M. fatua* aril exhibited dose dependent response to hot plate. Among different extract doses, significantly higher analgesic activity was noticed at 400mg/kg of extract.

At 0th min., there was no significant difference in the analgesic effect was found between different experimental groups of mice. Also after 30 min., standard drug pentazocine exhibited significantly high latency time of 4.5 sec followed by aril extract at 400mg/kg with reaction time of 3.94 sec was recorded. After 60 min., the order of the reaction time to hot plate was found to be Pentazocine group (5.61 sec) > 400mg/kg extract group (4.625 Sec) >200mg/kg extract group (4.125 Sec) > 100mg/kg extract group (3.605 Sec) > control group (2.075 Sec) .

There was gradual decrease in the reaction time was observed in all three extract groups except Pentazocine and control group. At 120th min., maximum latency time of 4.05 sec to hot plate was recorded in mice treated with 400mg/kg dose. However, standard drug Pentazocine showed significantly higher analgesic effect with a high latency period of 7.05 sec.

Table 33. Analgesic activity of the Ethyl acetate extract of *M. fatua* by tail immersion method in rats.

Group	Time Interval (Min.)				
	0	30	60	90	120
	Reaction time (Sec)				
1. Control	1.97±0.05^{a}	2±0^{d}	2±0^{d}	2±0^{e}	2±0^{c}
2 Pentazocine (10mg/g)	1.87±0.09^{a}	4.87±0.25^{a}	5.75±0.1^{a}	6.75±0.14^{a}	7.05±0.1^{a}
3. MFAEA (100 mg/kg)	1.95±0.1^{a}	2.2±0.14^{d}	3±0^{c}	3.02±0.05^{d}	2.075±0.05^{c}
4. MFAEA (200 mg/kg)	1.92±0.09^{a}	2.52±0.05^{c}	3.3±0.08^{c}	3.5±0.08^{c}	2.47±0.05^{c}
5. MFAEA (400 mg/kg)	1.97±0.05^{a}	2.85±0.05^{b}	3.67±0.05^{b}	3.87±0.09^{b}	3.5±0.57^{b}
F -value	1.050	302.448	394.50	1686.29	256.963

Table 33. represents the analgesic activity of ethyl acetate extracts using tail immersion method in Wistar rats. Ethyl acetate extract of *M. fatua* aril exhibited dose dependent response to tail immersion method. Among different extract doses, significantly higher analgesic activity was noticed at 400mg/kg of extract.

At 0th min., there was no significant difference in the analgesic effect was found between different experimental groups of rats. Also after 30 min., standard drug pentazocine exhibited significantly high latency time for tail withdrawal response of 4.87 sec followed by aril extract at 400mg/kg with reaction time of 2.85 sec was recorded. An increasing trend in the period of tail withdrawal response was noticed till 90th min and thereafter decreased gradually. Pentazocine group (5.75 sec) > 400mg/kg extract group (3.67 Sec) >200mg/kg extract group (3.3 Sec) > 100mg/kg extract group (3 Sec) > control group (2 Sec) was the order of the reaction time for tail withdrawal response at 60th min.

There was gradual decrease in the reaction time was observed in all three extract groups except Pentazocine and control group. At 120th min., maximum latency time of 4.05 sec to hot plate was recorded in mice treated with 400mg/kg dose.

However, standard drug Pentazocine showed significantly higher analgesic effect with a high latency period of 7.05 sec.

Table 34. Effect of Ethyl acetate extract of *M. fatua* aril on carrageenan induced paw edema in rats.

Group	Paw edema volume (ml)				
	0 min.	30 min.	60 min.	120 min.	180 min.
1. Control	0.975±0.09[a]	1.17±0.1[a]	1.30±0.008[a]	1.40±0.08[a]	1.325±0.09[a]
2.Diclofenac (10mg/kg)	0.53±0.05[b]	0.70±0[c]	0.64±0.04[b]	0.50±0[c]	0.40±0.08[c]
3. Extract (200mg/kg)	0.45±0.05[bc]	0.82±0.0[bc]	0.72±0.01[b]	0.675±0.05[b]	0.625±0.09[b]
4. Extract (400mg/kg)	0.32±0.05[c]	0.855±0.5[b]	0.70±0[b]	0.600±0[bc]	0.50±0.08[bc]
F- value	69.455	39.175	177.46	294.091	88.526

After 3 hrs, there was reduction in the swelling of paw volume (ml) from 1.325±0.09 ml in control to 0.625±0.09 and 0.50±0.08ml in rats treated with ethyl acetate extract of aril at the concentration of 200 and 400mg/kg respectively **(Table 34).**

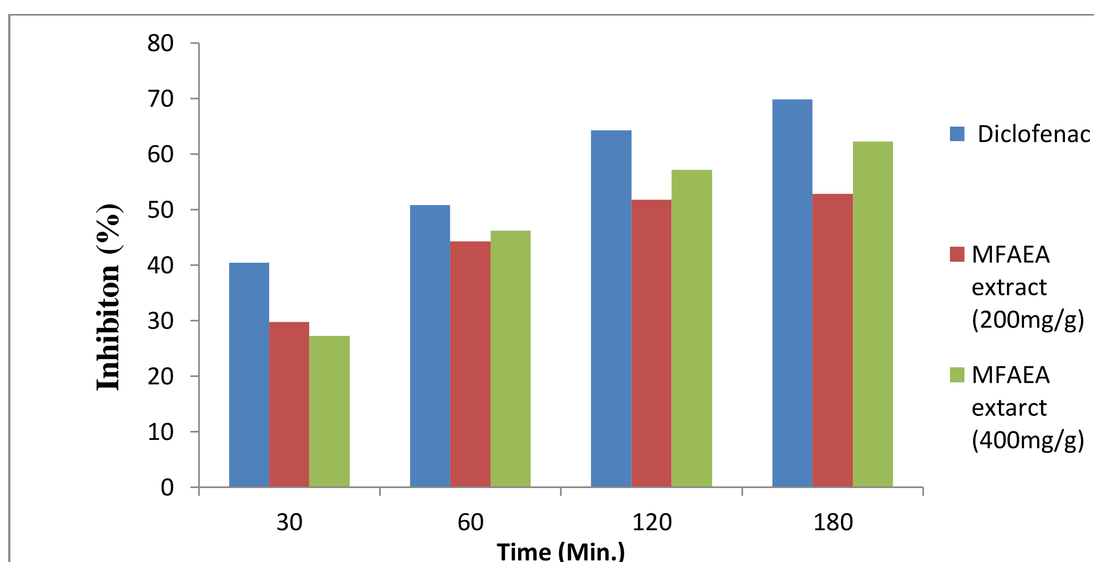


Figure 12. Effect of ethyl acetate extracts of *M. fatua* aril on carrageenan induced paw edema in rats.

Fig 12. indicates the inhibition shown by of *M. fatua* aril ethyl acetate extract towards the carrageenan induced paw edema in rats. At the end of 3 hrs, aril ethyl acetate extract at 200mg/kg dose exhibited a good inhibition of about 52.83% while 400mg/kg dose exhibited 62.26% of inhibition against paw oedema which is comparable with the Standard diclofenac with inhibition of 69.81%.

Table 35. Anti-diabetic activity of ethyl acetate extracts of *M. fatua* kernel control and Experimental rats.

Group	Day 0	Day 5	Day 10	Day 15
1. Control	77.83±0.17[a]	76.75±0.95[a]	76.14±1[a]	77±0.81[a]
2. STZ	279.77±0.51[a]	287.25±0.57[e]	291±0.81[e]	296.5±0.57[e]
3. STZ+ Std Glibenclamide	279.5±0.89[a]	100±0.81[b]	87.25±0.5[b]	79.75±0.5[b]
4. STZ+ MFKEA (200Mg/Kg)	280.2±0.29[a]	228.25±0.5[d]	193.5±0.57[d]	180.5±0.57[d]
5. STZ+ MFKEA (400Mg/Kg)	280±0.81[a]	190.05±0.42[c]	163.75±0.5[c]	134.5±0.5[c]
F- value	70265.57	67696.81	58000	87172.84

Table 35. indicates the effect of ethyl acetate extract of *M. fatua* kernel on fasting blood glucose level of STZ induced hyperglycaemic rats. A steep hike in the fasting blood glucose level was observed soon after the intraperitonial injection of Streptozotocin (STZ) was observed on 0th day. There was steep decrease in the fasting blood glucose level was recorded in the rats administered with standard drug, Glibenclamide (100±0.81 mg/dl) and which reached to normal level at the end of 15 days. On 5th day onwards, there was gradual decrease in fasting blood glucose level was registered in STZ- induced diabetic rats upon treatment of ethyl acetate extract (200mg/kg b.w.). Treatment with ethyl acetate extract STZ- induced diabetic rats exhibited 134.5mg/dl of fasting blood glucose level at the end of 15th day, which was comparable with Std Glibenclamide at the dose of 0.5mg/kg body weight.

Table 36. Effect of ethyl acetate extracts of *M. fatua* kernel on TC, TG, HDL, LDL, VLDL level in serum of control and Experimental rats.

Group	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
1. Control	58.67±0.57[c]	62.33±1.38[c]	39.8±0.57[a]	6.4±1.11[d]	12.47±0.11[d]
2. Triton-X-100	96.33±2.51[a]	106.67±1.24[a]	18.33±0.57[d]	56.67±3.2[a]	21.33±0.30[a]
Triton+ MFKEA (400mg/g)	66.67±0.57[b]	64±0.81[c]	24±1[c]	29.27±0.6[b]	13.4±0.20[c]
Triton+Atorvastatin (10mg/kg)	61.67±0.57[c]	80.67±0.81[b]	32.8±1.70[b]	12.47±1.2[c]	15.6±0.20[b]
F- value	490.72	1017.61	174.67	449.19	1017.61

There was significant increase in TC, TG, LDL and VLDL level was observed in Triton-X-100 induced rats compared to normal rats **(Table 36).** Similarly a significant decrease in TC, TG, LDL and VLDL levels were observed with oral administration of ethyl acetate extract of *M. fatua* kernel (400mg/g) in Triton- X-100 induced hyperlipedemic rats. Also there was significantly increase in the HDL level in the serum of hyperlipidemic rats upon administration of extract (24mg/dl) and standard drug (32.8mg/dl) compared to positive control group (18.33mg/dl).

Table 37. Showing thin layer chromatography of *M. fatua* kernel and aril extracts.

Sample	Stationary Phase	Mobile Phase	Spots	Colour of bands Under UV –C (280-10nm) light	R_f value
Kernel hexane extract		Benzene: Ethyl acetate : Hexane:: 4:1:1	1	Pinkish brown	0.150
			2	Purple	0.254
			3	Brownish yellow	0.491
		Toluene: Hexane: Ethyl acetate:: 8:2:4	1	Pinkish brown	0.135
			2	Purple	0.186
			3	Brownish yellow	0.271
			1	Brownish yellow	0.235
			2	Violet	0.411

<table>
<tr><td rowspan="5">Aril ethyl Acetate Extract</td><td rowspan="15">Silica gel G</td><td rowspan="5">Toluene: Ethyl acetate:: 10:1</td><td>3</td><td>Light violet</td><td>0.505</td></tr>
<tr><td>4</td><td>Brownish black</td><td>0.635</td></tr>
<tr><td>5</td><td>Violet</td><td>0.741</td></tr>
<tr><td>6</td><td>Light violet</td><td>0.811</td></tr>
<tr><td>7</td><td>Blackish brown</td><td>0.847</td></tr>
<tr><td rowspan="10">Aril chloroform extract</td><td rowspan="5">Hexane: Ethyl acetate::1:1</td><td>1</td><td>Brownish yellow</td><td>0.254</td></tr>
<tr><td>2</td><td>Brownish yellow</td><td>0.450</td></tr>
<tr><td>3</td><td>Brownish yellow</td><td>0.686</td></tr>
<tr><td>4</td><td>Violet</td><td>0.862</td></tr>
<tr><td>5</td><td>Violet</td><td>0.941</td></tr>
<tr><td rowspan="5">Toluene: Ethyl acetate:: 20:1</td><td>1</td><td>Violet</td><td>0.277</td></tr>
<tr><td>2</td><td>Violet</td><td>0.349</td></tr>
<tr><td>3</td><td>Violet</td><td>0.493</td></tr>
<tr><td>4</td><td>Violet</td><td>0.590</td></tr>
<tr><td>5</td><td>Violet</td><td>0.759</td></tr>
</table>

TLC of hexane extract of *M. fatua* kernel revealed the presence of 3 spots having R_f values of 0.150, 0.254, 0.491 when a solvent phase of Benzene: Ethyl acetate: Hexane (4:1:1) was used as shown in **Table 37.** In another mobile phase i.e., Toluene: Hexane: Ethyl acetate (8:2:4) also 3 spots were observed with R_f value of 0.135, 0.186, 0.271.

TLC of ethyl acetate extract of *M. fatua* resulted in 7 spots with R_f values of 0.235(Brownish yellow), 0.411(Violet), 0.0505(Light violet), 0.635(Brownish black), 0.741(Violet), 0.811(Light violet) and 0.847 (Blackish brown) respectively in the mobile phase of Toluene: Ethyl acetate (10:1). All the 7 spots were prominent and clear.

Chloroform extract of *M. fatua* aril resulted in 5 spots of R_f value 0.254, 0.450, 0.686, 0.862 and 0.941 respectively when the mobile phase was Hexane: Ethyl acetate (1:1). In another mobile phase of Toluene: Ethyl acetate (20:1) TLC resulted in the 5 violet coloured spots with R_f value of 0.227, 0.349, 0.493, 0.590 and 0.759 respectively.

Table 38. FTIR spectral peak values and functional groups obtained for the different fractions of *Myristica fatua* aril ethyl acetate extracts.

Sample	Peak values	Functional groups
Fraction-1	3322	-OH stretching
	1626	-C=O stretching
	1450	$-CH_2$&$-CH_3$ deformation
	1224	-O-C stretching
	721	$-CH_2-$ rocking
Fraction- 2	3344	-OH stretching
	1732	-C=O stretching
	1448	$-CH_3$ deformation
	1371	$-CH_3$&$-CH_2$ deformation
	1236	O-C –stretching
	725	Weak $–CH_2$ rocking
Fraction-3	2922	=C-H stretching
	2852	-C-H stretching
	1727	-C-O stretching
	1226	-C-O-C stretching

The FTIR spectroscopic analysis showed the presence of functional groups **(Table 38. & fig 13a-13c).** The fraction -1 of *Myristica fatua* aril ethyl acetate extract showed IR absorption bands at absorption bands at 3322 cm^{-1} (for –OH stretching), 1450 cm^{-1} (CH_2&$-CH_3$ deformation), 1224 cm^{-1} (-O-C stretching) and 721 cm^{-1} ($-CH_2-$ rocking) indicating the presence of acids while 3344 cm^{-1}(-OH stretching), 1732 cm^{-1} (-C=O stretching), 1448 cm^{-1}($-CH_3$ deformation), 1371 cm^{-1}($-CH_3$&$-CH_2$ deformation), 1236 cm^{-1}(O-C –stretching) and 725 cm^{-1}(Weak $–CH_2$ rocking) were the IR absorption bands for the fraction -2 indicating presence of acids. The Fraction -3 of *Myristica fatua* aril ethyl acetate extract showed characteristic IR absorption bands at 2922 cm^{-1} (for =C-H stretching), 2852 cm^{-1} (-C-H stretching),1727 cm^{-1}(-C-O stretching) and 1226 cm^{-1}(-C-O-C stretching) indicating the presence of terpene compounds.

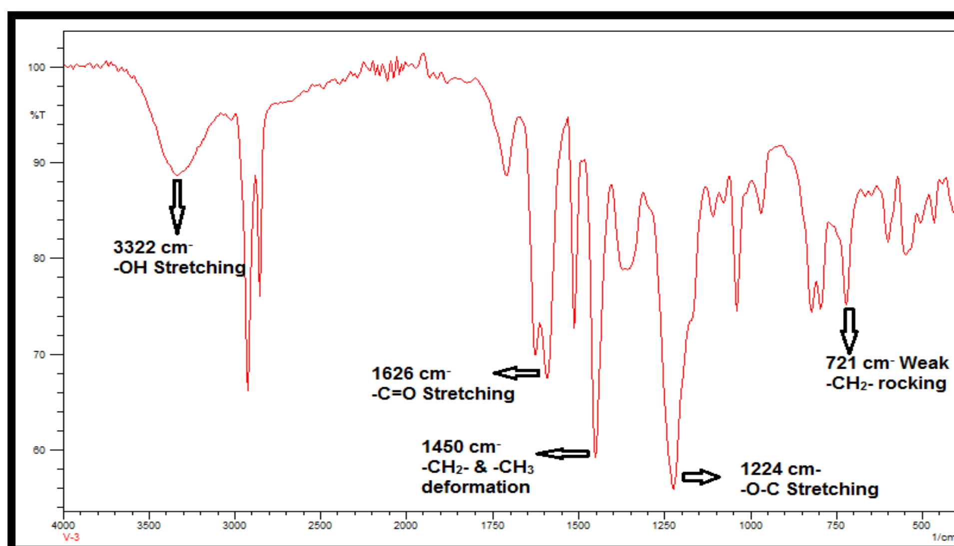


Fig.13a. I. R. Spectrum of aril Ethyl acetate extract Fraction- 1

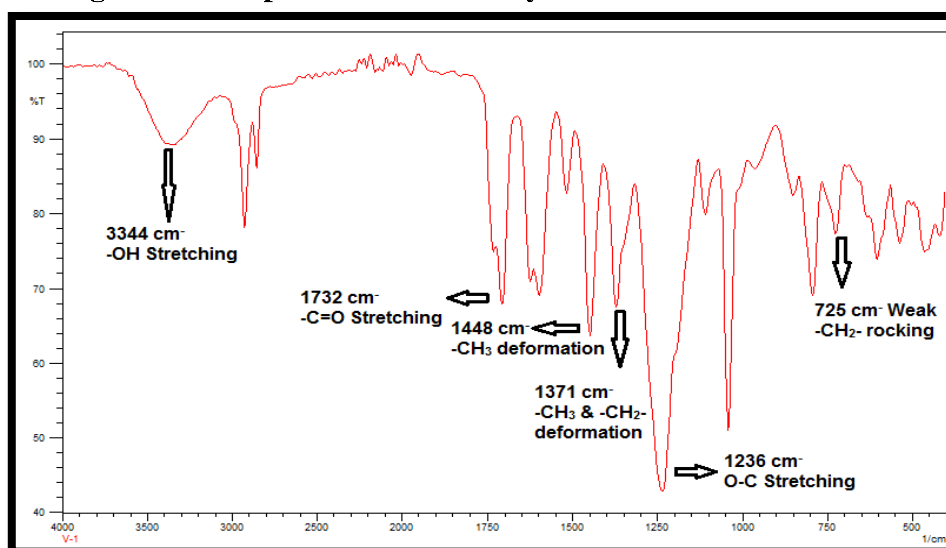


Fig.13b. I. R. Spectrum of aril Ethyl acetate extract Fraction- 2.

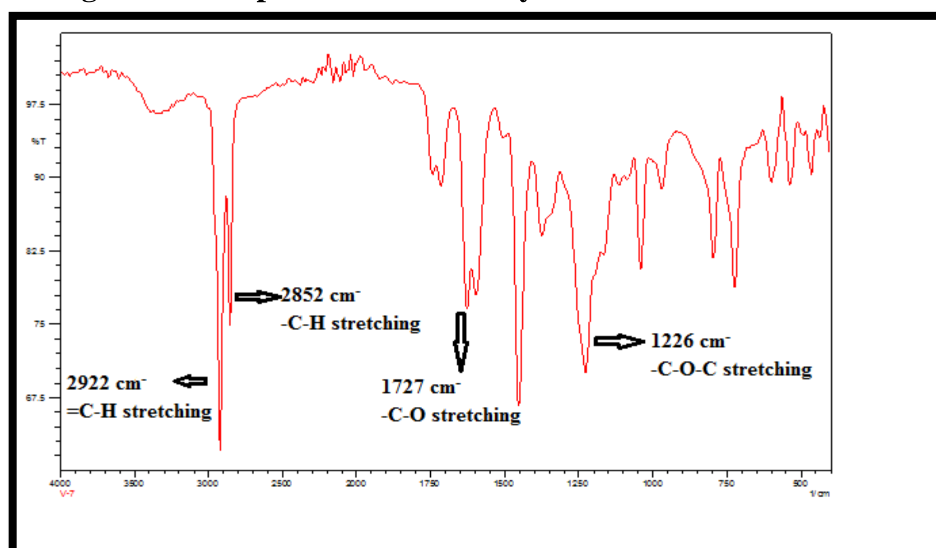


Fig.13c. I. R. Spectrum of aril Ethyl acetate extract Fraction- 3

REFERENCE

1. A. O. A. C. (1990) Official Method of Analysis 15th Edition, Association of Official Analytical Chemists Washington, D. U.S.A.

2. Ajish A D, Vagdevi H M, Asha K & Jayanna N D 2015 Potential antimicrobial, anthelmintic and antioxidant activities of Myristica dactyloides Gaetrn bark. Int J Pharm Pharm Sci 7:177-122.

3. Anonymous 1962 The wealth of India, raw materials. Council for Scientific and Industrial research, New Delhi, 6: 474-479.

4. Banerjee D, Buari A K, Guha R K, Bandyopadhyay S K & Chattopadhyay S 2008 Healing properties of malabaricone B and malabaricone C, against indomethacininduced gastric ulceration and mechanism of action. Eur. J. Pharmacol. 127:4140-4141.

5. Banerjee D, Maity B, Bauri A K, Bandyopadhyay S K & Chattopadhyay S 2007 Gastroprotective properties of Myristica malabarica against indomethacin- induced stomach alceration: a mechanistic exploration. J Pharm Pharmacol 59: 1555- 1565.

6. Bhat R P & Kaveriappa K M 1998 Chemical composition of kernel and mace of Myristica fatua var. magnifica (Beddome) Sinclair- A threatened taxon of the Western Ghats, India. Ad. Plant Sci. 11:235-237.

7. Biswas M, Kar B, Bhattacharya S, Kumar R B S, Ghosh A K & Haldar P K 2011 Antihyperglycemic activity and antioxidant role of Terminalia arjuna leaf in streptozotocin-induced diabetic rats. Pharm Biol. 49:335-340.

8. Choi N H, Choi G J, Jang K S, Choi Y H, Lee S O, Choi J E & Kim J C 2008 Antifungal Activity of the Methanol Extract of Myristica malabarica Fruit Rinds and the Active Ingredients Malabaricones against Phytopathogenic Fungi. Plant Pathol. J. 24: 317-321.

9. Choi N H, Kwon H R, Son S W, Choi G J, Choi Y H, Jang K S, Lee S O, Choi J E , Luu Hoang NGOC & Jin- Cheol Kim 2008 Nematicidal activity of malabaricones isolated from Myristica malabarica fruit rinds against Bursaphelenchus xylophilus. Nematology 10: 801-807.

10. Choi N H, Kwon H R, Son S W, Choi G J, Choi Y H, Jang K S, Lee S O, Choi J E, Ngoc L H & Kim J C 2008 Nematicidal activity of malabaricones isolated from Myristica malabarica fruit rinds against Bursaphelenchus xylophilus. Nematology 10: 801- 807.

11. Cooray N F, Jansn E R, Wimalasena S, Wijesekera T P & Nair M B 1987 Acylresorcinols from seed kernels of Myristica dactyloides. Phytochemistry 26: 3369-3371.

12. Dasappa, Ram J 1999 Conservation of hot spots of rare, endangered and threatened species in Western Ghats. My Forest 35:201-5.

13. Desrivot J, Waikedre J, Cabalion P, Herrenknecht C, Bories C, Hocquemiller R & Fournet A 2007 Antiparasitic activity of some new Caledonian medicinal plants. J Ethnopharmacol 112: 7-12.

14. Dir.90/496/CEE

15. Dubois M K, Gilles J K, Hamilton P A & Smith F 1951 A colorimetric method of determination of sugar .Nature 167:168.

16. Duggal S P & Kartha A R S 1956 Antioxidants for edible oils and fats from seeds of Myristica species. Indian J. Agric. Sci. 26: 391-399.

17. Franzotti E M, Santos C V F, Rodrigues H M S L, Mourao R H V, Andrade M R & Antoniolli A R 2000 Anti-inflammatory, analgesic activity and acute toxicity of Sida cordifolia L. (Malva-branca). J. Ethnopharmacol. 72: 273-277.

18. Friedewald W T, Levy R I & Fredrickson D S 1972 Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem. 18:499-502.

19. Gamble J S 1967 Flora of the Presidency of Madras, Botanical Survey of India,

Calcutta. 20. Ghosh M N. Fundamentals of Experimental pharmacology. 3rd Edition. Kolkata: Hilton and Company; 2005.

21. Gokhale S B, Joshi P V, Tatiya A U, Bakliwal S R & Fursule R A 2004 Some neglected spices in India. Nat. prod. radiance 3: 343–346.

22. Gupta M, Mazumder U K, Gomathi P & Selvan V T 2006 Anti-inflammatory evaluation of leaves of Plumeria acuminate. BMC Complement Altern Med. 6: 1472-6882.

23. Hedge J E & Hofreiter B T. Carbohydrate Chemistry. 17th ed. New York: Academic Press; 1962.

24. Herath H M T B & Priyadarshani A M A 1996 Two lignans and an aryl alkanone from Myristica dactyloides. Phytochemistry 42:1439-1442.

25. Herath H M T B & Priyadarshani A M A 1997 Lignans from Myristica dactyloides.

Phytochemistry 44: 699-703.

26. Hooker J D 1973 Flora of British India, Periodicals experts, Vivek Vihar, New Delhi. pp. 102.

27. Indrayan A K, Sharma S, Durgapal D, Kumar N & Kumar M 2005 Determination of nutritive value and analysis of mineral elements for some medicinally valued plants from Uttraranchal. Current Sci., 89(7): 1252-1255.

28. Jackson M C 1962 Soil ChemicalAnalysis. Prentice Hall Inc . Engle Wood Cliffs , New Jersy, USA.

29. Joshi B S, Ravindranath K R & Viswanathan N 1977 Structure and stereochemistry of attenol, a new lignan from Knema attenuata (Wall.) Warb. Cell Mol Life Sci 20: 422-423.

30. Khanom F, Hiroshikayahara & Tadasa K 2000 Superoxide- Scavenging and prolyl endopeptidase inhibitory activities of Bangladeshi indigenous medicinal plants. Biosci. Biotechnol. Biochem. 64: 837-840.

31. Kim J S, Kwon C S & Son K H 2000 Inhibition of alpha- glucosidase and alpha- amylase by Luteolin, a Flavonoid. Biosci. Biotechnol. Biochem 64:2458-2461.

32. Krishnamoorthy K 1960 Myristica swamps in the evergreen forests of Travancore. Indian For. 86: 314-315.

33. Kumar N S S, Herath H M T & Karunaratne V 1988 Arylalkanones from Myristica dactyloides. Phytochemistry 27: 465-468.

34. Kumar R K & D K Ved 2000 One Hundred Red Listed Medicinal Plants of Conservation Concern in Southern India, Bangalore, FRLHT.

35. Liyana- Pathiranan C M & Shahidi F. Antioxidant activity of commercial soft and hard wheat (Triticum aestivum L.) as affected by gastric pH conditions. J Agri Food chem 2005 53: 2433-40.

36. Lowrry O H, Rosebrough N J, Fan A L & Randall R J 1951 Protein measurement with the Folin phenol reagent. J Biol Chem, 73:193.

37. Mabberley D J 1990 The Plant-Book: A Portable Dictionary of the Higher Plants, Cambridge University Press, Cambridge. pp. 258-386.

38. Manjunatha B K, Hegde V, Abhilash N & Divakara R 2012 Evaluation of In-vitro Antioxidant and Invivo Hepatoprotective Potency of Myristica Malabarica. Res. J. Pharm., Biol. Chem. Sci. 3:1044-1052.

39. Manjunatha B K, Mankani K L, Mukunda S K, Divakara R, Sridar B K & Paul K 2011 Antioxidant and Hepato Protective Effect of Myristica malabarica seed Aril Extracts on Carbon Tetrachloride Induced Hepatic Damage. Global Journal of Biotechnology & Biochemistry 6: 25-30.

40. Maya K M, Zachariah J T, Krishnamurthy K S, Rema J & Krishnamoorthy B 2006 Fatty acids and leaf amino acids in Myristica fragrans and its wild taxa. Indian J Hortic. 63: 316-318.

41. National Committee for Clinical Laboratory Standards (NCCLS). 2000 Performance standard for antimicrobial disc susceptibility test. Approved standard NCCLS document M2-A7. Wayne Pa.

42. New OCED 425 guidelines. OCED Guidelines for testing animals. 2001 Dec 1; 26: 1-26.

43. Obadoni BO & Ochuko PO 2001 Phytochemical studies and comparative efficacy of the crude extract of some homeostatic plants in Edo and Delta states of Nigeria. Global J. Pure Appl. Sci., 8: 203- 208.

44. Oyaizu M. Studies on product on browning reaction prepared from glucose amine. Jpn. J.Nutr 1986; 44:307-15.

45. Padmanabhan P & Jangle N S 2012 Evaluation of In-vitro anti inflammatory activity of herbal preparation, a combination of four medicinal plants. Int. J Basic Appl. Med. Sc., 2:109-116.

46. Patro B S, Bauri A K, Mishra S & Chattopadhya S 2005 Antioxidant activity of Myristica malabarica extracts and their constituents. Agric Food Chem 53: 6912–6918.

47. Purushothaman K K, Sarada A & Connolly J D 1997 Malabaricones A-D, novel diarylnonanoids from Myristica malabarica Lam. (Myristicaceae). J. Chem. Soc. Perkin 5: 587–588.

48. Ramachandra T V, Chandran S M D, Joshi N V, Sooraj N P, Rao G R & Vishnu M 2012 Ecology of Sacred Kan Forests in Central Western Ghats. Sahyadri Conservation Series 15. pp. 1-103.

49. Sabulal B, Kurup R, Sumitha & George V 2007 Chemical composition of the Leaf Essential Oils of Myristica malabarica Lam. and Gymnacranthera canarica (King) Warb. J. Essent. Oil Res. 19:323-325.

50. Sawadogo, W.R., Boly, R., Lompo, M., Some, N., 2006. Anti-inflammatory, analgesic and antipyretic activities of Dicliptera verticillata. Intl J Pharmacol. 2(4): 435-438.

51. Schanderl, S H 1970 In: Method in Food Analysis Academic Press New York p 709.

52. Sen R, Bauri A J, Chattopadhyay S & Chatterjee M 2007 Antipromastigote activity of the malabaricones of Myristica malabarica (rampatri). Phytother Res. 21:592-595.

53. Sreenivasan B 1968 Component fatty acids and composition of some oils and fats. J. Am. Oil Chem. Soc. 45: 259-265.

54. Subha S, Vijayakumar B, Prudhviraj K, Aanandhi V M, Shankar M & Nishanthi M 2013 Pharmacognostic and preliminary phytochemical investigation on leaf extracts of Myristica dactyloides Gaertn. International Journal of Phytopharmacology 4: 18-23.

55. Taga M S, Miller E E & Pratt DE. Chia seeds as a source of natural lipid antioxidants. J Am Oil Chem Soc 1984; 61:928-31.

56. Talukdar A C, Jain N & Krishnamurty D S 2000 An isoflavone from Myristica malabarica. Phytochemistry 53:155-157.

57. Tandon, H L S.edn, 1993. Methods of Analysis of soils, plants, waters and fertilizers. Fertilizer development and consultant organization, New Delhi, India.

58. Tillekeratne L M V, Jayamanne D T & Weerasooria K D V 1981 Chemical constituents of Myristica dactyloides. J. Natn. Sci. Coun. Sri Lanka 9:251-25.

59. Trinder P. 1969 Ann. Clin. Biochem. 6(24)1969.

60. Upudha A L, Rathnakar U P & Udupa S 2007. Antiinflammatory, Anti-pyretic and Analgesic effect of Tamrindus indica. Indian Drug, 44:466-470.

61. Vinayachandra & Chandrashekar K R 2014 Phenolic Contents of Knema attenuata Fruits and their Bioactive Potentials. Journal of Herbs, Spices & Medicinal Plants 20:183–195.

62. Vinayachandra, Shwetha R & Chandrashekar K R 2011 Larvicidal activities of Knema attenuata (Hook. f. & Thomson) Warb. (Myristicaceae) extracts against Aedes albopictus Skuse and Anopheles stephensi Liston. Parasitol Res. 109:1671-1676.

63. Viveka M R & Chandrashekar K R 2016 Antioxidant and antibacterial activities of Myristica fatua var. magnifica (Beddome) Sinclair. Asian J Pharm Clin Res 9: 235-239.

64. Winter C A, Riely E A & Nuss G W 1962 Carrageenan induced edema in hind paw of the rats as assay for anti-inflammatory drugs. Proc Soc Exp Biol Med. 111: 544-547.

65. Zachariah T J, Leela N K, Maya K M, Rema J, Mathew P A, Vipin T M & Krishnamoorthy B 2008. Chemical composition of leaf oils of Myristica beddomeii (King), Myristica fragrans (Houtt.) and Myristica malabarica (Lamk.) Journal of Spices and Aromatic Crops 17: 10–15.

66. Zichen J, Mengcheng T & Jianming W 1999 The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem 64:555-9.